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Proposals for the Development, Composition, and Routine Use of System Suitability Standard Mixtures in Support of Chromatographic Screening for Organic Extractables and Leachables[a]

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ABSTRACT

Leachables present in packaged drug products or released from medical devices can adversely affect patient health and safety. Thus, packaged drug products are screened for unspecified leachables, and packaging system or medical device extracts are screened for unspecified extractables as potential leachables, a process known as non-targeted analysis (NTA). Screening methods for organic extractables and leachables typically employ chromatography to separate analytes and information-rich detectors [such as mass spectrometry (MS)] to detect, identify, and quantify them.

Chromatographic screening methods are generally qualified to establish that they are suitable for their intended use. When the qualified methods are implemented during extractables and leachables studies, system suitability testing is conducted during each chromatographic run to ensure that the method performs properly at the time of use.

System suitability testing in extractables and leachables screening requires a standard mixture of relevant compounds that themselves are extractables and leachables. To facilitate consistent analytical performance across laboratories and to standardize system suitability testing, a standardized system suitability mixture (meaning a mixture with specified constituents), used by all practitioners, is necessary.

Based on several scientific and practical considerations, USP is developing a set of system suitability reference standards for the most commonly employed hyphenated chromatographic screening methods, such as gas and liquid chromatography with mass spectrometric detection (GC/MS and LC/MS). In this article, the USP approach to reference mixture development is discussed; the compositions of the reference standard mixtures are disclosed, discussed, and justified; and typical chromatograms are provided. USP is seeking feedback from stakeholders on the proposed mixtures. The article also discusses other opportunities for development of reference standards and reference standard mixtures to support extractables and leachables testing (e.g., calibration mixtures, individual reference standards for “hard-to-find” extractables, and leachables, etc.).

1. INTRODUCTION

The purpose of this *Stimuli* article is to discuss reference standard mixtures and to disclose, discuss, and justify the system suitability standard mixtures the USP is developing for the commonly employed hyphenated chromatographic methods used for organic extractables and leachables screening.

1.1 What Are Extractables and Leachables and Why Are They Important?

From the time it is manufactured, from starting materials to the point where it is administered to patients, a drug product (and/or its precursors) will be in contact with manufacturing components, packaging systems, and administration devices. Thus, it is almost certain that drug products (or their precursors) will chemically interact with their manufacturing systems, packaging, and/or administration devices via the transfer of chemicals.

Leachables are substances that have transferred (leached) from these items into the drug product under its typical conditions of manufacturing, storage, and use. A related term, extractables, also refers to substances that have been leached but unlike leachables, extractables are leached (extracted) by, and accumulate in, solutions other than the drug product. This leaching occurs under laboratory conditions of contact that differ from and are generally more aggressive than the clinical conditions of contact between the item and the drug product.

The existence of leachables is important not just from the perspective of product purity (as leachables are drug product impurities that generally serve no useful purpose and detract from a product's purity) but more importantly from the perspective of the leachables' effect on other drug product key quality attributes. For example, reactive leachables and leachables that affect the drug product's chemistry (e.g., pH) can adversely affect the product's potency and stability. Unsafe leachables adversely affect user's health and safety. Unpleasant leachables adversely affect a drug product's aesthetics (e.g., discoloration and odor), and poorly soluble or complexing leachables can generate particulates.

Drug products are tested for leachables (and relevant items are tested for extractables as potential leachables) so that the effect of leachables on the drug product's key quality attributes can be inferred, as required by global regulatory agencies (*1*–*2*) and discussed by standards-setting (*3*–*7*) and scientific organizations (*8*–*10*).

1.2 The Role of Screening in Extractables and Leachables Testing

There are two analytical strategies for testing an extract to discover, correctly identify, and accurately quantify extractables above an established analytical evaluation threshold (AET), or when testing a drug product for leachables for the same purpose. In the first strategy, targeting, analytes are specified up-front, and the test method must quantify the targeted analytes if they are present in the sample at levels above the AET. Thus, targeting answers the questions, "are these specified compounds present in the sample at a level above the AET and, if they are, what is their concentration?" In the second strategy, screening or non-target analysis (NTA), it is presumed that extractables or leachables are present in the test sample, although there is no presumption about the identity of these analytes. In this case, screening answers the more general questions, "what compounds are present in this sample above the AET and what is their identity and concentration?" Given these different purposes, it is logical that while screening methods and target methods may share the same general analytical approach (for example, GC/MS may be suitable for both targeting and screening for semi-volatile organic compounds), they likely differ in key operational parameters.

All things being equal, it is obvious that the analytical process of targeting specified extractables and leachables produces the highest quality data based on the rigorous validation of targeting methods. However, targeting applied to extractables and leachables would require that all possibly relevant extractables or leachables be specified up-front. Although this is a clear and noble goal, there are practical realities that make this a nearly impossible task. Thus, securing a complete extractables and leachables profile requires, almost without exception, screening (or NTA), alone or in concert with target analysis.

1.3 Chromatographic Methods for Extractables and Leachables Screening for Organic Compounds

Potential organic extractables and leachables are a large population of structurally, functionally, and chemically diverse compounds. Although chromatographic methods are most commonly employed for testing samples for organic extractables and leachables, no single chromatographic method is sufficiently broad in scope to cover all the possibilities. Thus, testing of samples for organic extractables is based on an analytical strategy consisting of several individual techniques, depending on the volatility of the analytes. For example, gas chromatography with headspace-gas sampling (HS-GC) is commonly employed to address primarily volatile organic analytes, gas chromatography (GC) is employed to address primarily semi-volatile analytes and liquid chromatography (LC) is employed to address primarily non-volatile analytes. Thus, various overlapping but orthogonal chromatographic separation methods are used to encompass the widest possible range of organic substances, specifically considering the substances' volatility. The orthogonal aspect of the methods provides the widest breadth, whereas the overlap reduces the possibility of there being "gaps" between the methods (*11*) (*Figure 1*).

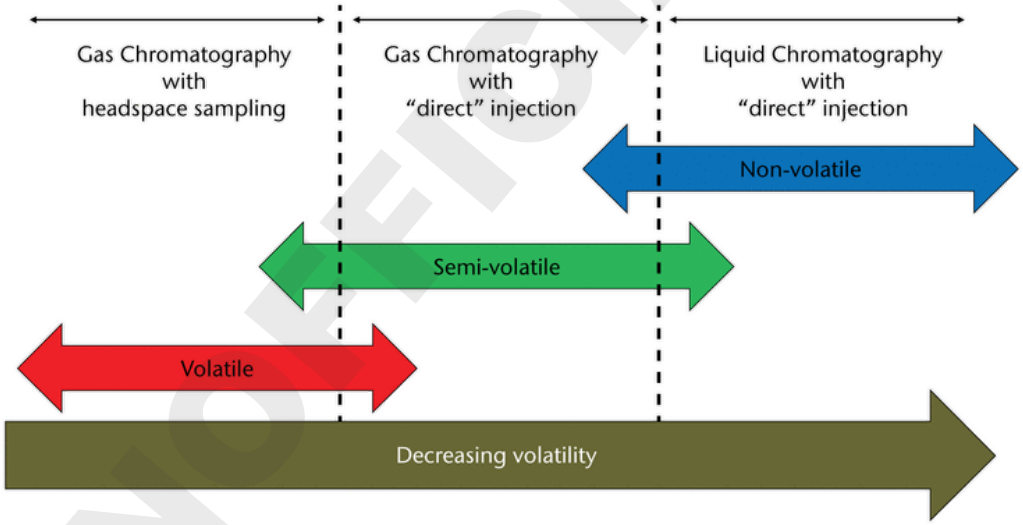

Figure 1. Application of chromatographic methods in extractables and leachables screening.

1.4 The Role of System Suitability Testing in Extractables and Leachables Screening

Chromatographic methods used for extractables or leachables screening must be suited for this intended purpose. Such methods are qualified as being suited for their intended use by establishing the methods' key performance attributes and comparing those attributes with necessary

performance expectations (acceptance criteria). Given the large number of potential extractables and leachables, screening methods are qualified using representative compounds. This contrasts with targeted methods, which are typically validated using the specified target compounds.

The fact that a screening method is qualified does not ensure that data of the requisite quality will be generated each time the method is implemented. Mistakes in analytical solution preparation can occur, column performance can decline, detectors can become dirty, injection liners can be contaminated, etc. Furthermore, there are multiple inherent sources of variation in the implementation of an analytical method such as different analytical instruments, reagent quality, and environmental conditions. Recognizing these realities, test methods are typically requalified, to a more limited extent than the initial qualification at the time of use; that is, the test method is established to be capable of producing data of the requisite quality each and every time it is used (intra-run) and during the course of its use (inter-run). This process of qualification at time of use, which is logically less intensive and extensive as the initial method qualification, is termed system suitability testing. A method has been established to be suitable for producing data of the requisite quality when system suitability testing has been performed and all performance expectations (acceptance criteria for system suitability) have been met at the beginning, throughout, and at the end of an analytical run.

System suitability addresses three performance aspects of a method at its time of use:

1. That the method has been set up and implemented properly
2. That the method as set up can perform at the same level it performed at during its qualification
3. That the method has performed acceptably throughout its use

System suitability does not establish whether a method is well suited for a particular application or not. Thus, a method that is not fit for purpose can meet the system suitability requirements and therefore be deemed to be operating properly. That is, system suitability does not define or extend a method's fundamental capabilities but rather simply establishes that at a particular point in time the method performed in the manner it was qualified to perform. Success in system suitability is not always reflected as success in the screening process, not because the system was improperly set up and implemented but rather because the method was poorly designed for a particular application.

System suitability for screening methods is established by testing a set of representative compounds as the universe of potential compounds that the methods must address is large. This set of representative compounds is termed as system suitability mixture.

1.5 Attributes of Efficient and Effective System Suitability Test Mixtures

Although system suitability assessment is essentially "qualification at time of use", one recognizes that system suitability assessment is not a process that involves repeating the method's complete qualification each time a method is implemented, as doing so is excessive and inconsistent with system suitability's intent. Thus, in designing an effective and efficient system suitability assessment process, one must accomplish two objectives:

1. Identify performance characteristics that were assessed during qualification, and which are necessary and appropriate to reassess at the time of use. System suitability assessment should focus on those minimum number of performance characteristics, which individually and in aggregate, demonstrate that the three performance aspects noted previously were achieved.
2. Establish the most efficient means of experimentally addressing those performance characteristics by performing the fewest number of tests, the fewest types of tests, and using the smallest set of reference compounds. This is the case because efficient time-of-use system suitability assessment can ill afford time-consuming and redundant tests performed on an excessively and unnecessarily large group of reference compounds.

These points are important in establishing the composition of a system suitability test mixture as the mixture must minimally contain a sufficient number and variety of compounds to support performance verification while at the same time avoiding unnecessary replication. This second point is important not only in terms of the practicality of time (for example, it is inefficient to perform 15–20 injections to address system suitability when the entire analytical run might only consist of 15–20 samples) but also in terms of being able to meet system suitability acceptance criteria (increasing the number of tests increases the likelihood of producing contradictory, and failing, system suitability test results).

Hypothetically, there is an optimal number of reference compounds (of varying structure and physicochemical properties) in a system suitability mixture. Too few compounds do not adequately address all of the appropriate performance characteristics, whereas too many compounds are inefficient and increase the possibility of producing contradictory, and false negative results.

An appropriate starting point for establishing the composition of a system suitability test mixture is establishing the performance characteristics and attributes that will be considered. System suitability parameters that are specifically relevant to extractables and leachables screening methods include:

- Sensitivity, as the method's limit of quantification must be less than or equal to the AET
- Specificity, the ability to assess unequivocally the analyte in the presence of other constituents that can be expected in the sample
- Accuracy, taken as the ability to produce an estimated concentration that is comparable to the true value (e.g., a measured concentration in a spiked extract that is comparable to the spiked amount)
- Linearity, the ability to establish a linear relationship between analyte response and concentration over a sufficiently broad range of concentrations
- Chromatographic performance (e.g., resolution, linked to specificity)
- System precision (injection-to-injection precision, inter-run precision)

Furthermore, certain performance characteristics in system suitability testing can be addressed with just one compound, leveraging the concept that if a performance characteristic that was qualified using multiple compounds is established as being adequate for a single compound

in system suitability assessment, then the set up that produced the single compound's acceptable data will also produce acceptable data for all compounds addressed during qualification. Performance characteristics of this type include dynamic range (linearity), sensitivity, accuracy, and precision.

It is efficient and effective to use critical and diagnostic substances that challenge method performance during qualification as members of a system suitability mixture. For example, if the system suitability question of "is the response large enough?" is addressed for a poorly responding member of the qualification mixture, then this member becomes a diagnostic substance, as surely it will be among the first substances to fail this acceptance criterion. If the answer to the question for a poor responding substance is "yes, the method is sensitive enough", then this answer will likely be yes for all of the other substances. If the answer to this question is "no" for a poor responding substance, then a method failure has been identified. Similarly, the process question "are the correct identities obtained?" could be addressed by considering a substance that is particularly challenging to identify. Presumably, if the processing of a method response produces the right identity for a very difficult substance to identify, then a similar outcome would be obtained for the more easily identified substances using the same processing of response. A similar discussion is relevant for linearity; that is, rather than establishing linearity for several compounds, if linearity is (or is not) established for a diagnostic compound then the same outcome would be obtained for the other compounds.

Lastly, the question "is the method's chromatographic efficiency adequate (i.e., is the method specific)?" could be answered by identifying a critical pair of compounds, likely eluting in the middle of a chromatogram. Establishing the resolution between the individual substances that make up the critical pair provides the means for assessing whether adequate efficiency (specificity) was achieved across the entire breadth of the analytical method.

Given this discussion, one could envision a suitability mixture consisting of a minimum of six substances (*Figure 2*). Although this example establishes the minimum number of substances, it does not necessarily reflect the optimum number of substances. Two substances in the mix, anchor compounds, would establish the range or breadth of the method, with one substance establishing the method's minimum starting point (the first eluting substance that is detectable by the method), and the other substance establishing the method's maximum ending point (the last eluting substance that is detectable by the method).

In addition to these two anchor substances, the suitability mixture will contain two substances that serve as the critical pair to address chromatographic efficiency (e.g., resolution). As one pair of substances is to be used to represent the entire chromatographic method, the pair would typically elute near the middle of the chromatogram.

Additionally, it is recognized that screening is not accomplished via the application of a single method but rather multiple orthogonal and overlapping methods. Thus, it is useful if the system suitability mixtures for the individual methods contain overlapping substances, that is, substances that are detectable by two or more methods. If these "overlapping" compounds were the anchor compounds, they would establish both the method's breadth and overlap.

Lastly, the mixture would contain a substance with a low magnitude of response (a low responsive substance or a substance in a low amount) to address sensitivity and a poorly responding substance (information content of the response) to address identification. These compounds test the ability of the method (and its operators) to produce data that can be interpreted, producing accurate concentrations and correct identifications.

The confirmation of accuracy and precision do not require additional reference substances but rather could be based on reference substances that serve multiple purposes.

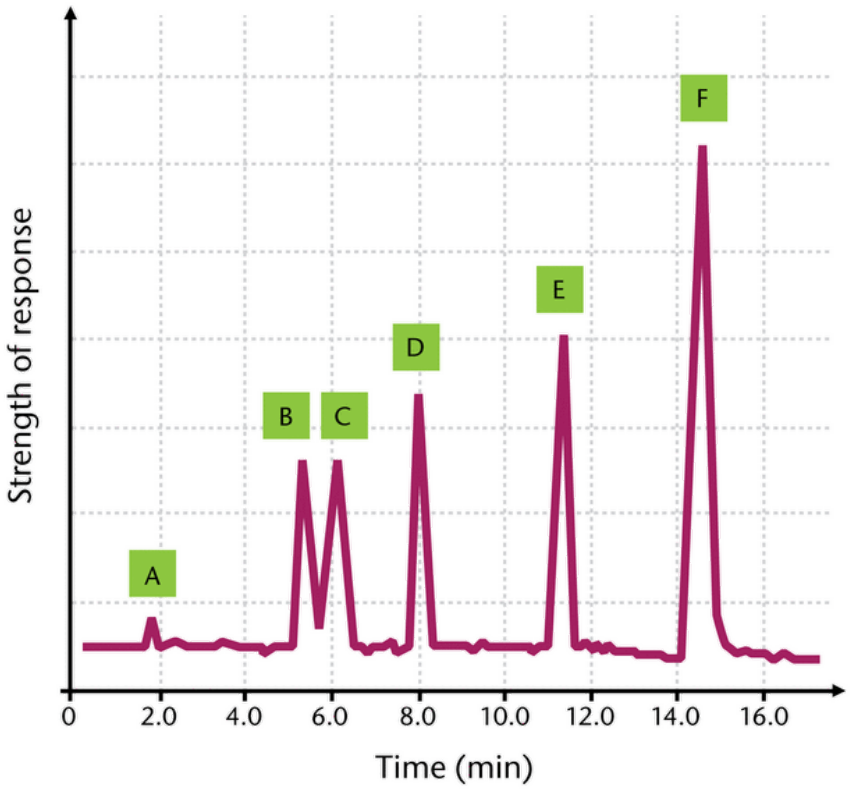

Figure 2. Sketch chromatogram obtained for a system suitability mixture containing six members. The substances associated with peaks A and F are the anchor substances, confirming the breadth of the method. Substances associated with peaks B and C represent the critical pair, whose resolution establishes that the chromatographic efficiency is adequate. The substance associated with peak A also addresses method sensitivity (quantitation), whereas the substance associated with peak E addresses the method's ability to produce an interpretable mass spectrum (identification) (*11*).

A smaller number of substances in the suitability mix may suffice if one or more substances serve a dual function, for example, a single substance that is both one of the critical pairs and the sensitivity marker. Although such an approach may appear to be more efficient, it may be more difficult for dual-purpose substances to meet two separate acceptance criteria. Thus, a larger number of substances in the mixture may be necessary in certain circumstances.

Considering the above, appropriate standard mixtures could be developed by fulfilling the following functions:

- **Anchor compounds:** Compounds that are the earliest and latest eluting compounds, establishing the chromatographic breadth of the method
- **Sensitivity compounds:** One or more compounds that establish that the method is sufficiently sensitive for its purpose
- **Critical pair:** At least one pair of closely eluting compounds to establish the method's chromatographic efficiency and resolving power
- **Overlapping compound:** Compounds that are detectable by the method in question and a second screening method
- **Special purpose compounds**
  - **Data processing check, deconvolution:** Closely eluting compounds (perhaps the critical pair) that are particularly difficult to resolve based on their similar mass spectra. This function is more relevant for GC/MS, where deconvolution is practiced more commonly than in LC/MS.
  - **Data processing check, identification by spectral matching:** Compound(s) that are particularly difficult to identify correctly based on their mass spectra. This function is more relevant for GC/MS, due to the relative lack of commercially available mass spectral databases for LC/MS.
- **Compounds present to add chemical diversity:** Compounds that are present in the mixture to ensure that the mixture contains chemically diverse members
- **Compounds of potentially toxic substances:** Compounds whose presence in a test sample could pose a potential toxicological safety risk
- **Precision compounds:** Compounds that are used to establish the method's injection precision (beginning of run) and inter-run precision (throughout the run). Generally, multiple compounds chosen that elute throughout the chromatogram and are chemically diverse are used.

1.6 Extractables and Leachables System Suitability Mixtures for Chromatographic Screening

The USP recognizes the importance of system suitability testing in extractables and leachables screening, noting in *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems ⟨1664⟩* (*7*) that "System suitability tests and criteria should also be developed for each leachables method" and that "Chromatography-based analytical methods, such as those described in *Chromatography*

⟨621⟩, should include appropriate system suitability criteria for routine method evaluation, including tests for method linearity, precision, sensitivity, and specificity as appropriate". Considering test mixtures, USP notes that "These parameters should be evaluated with an appropriately constituted test mixture(s) each time the quantitative leachables method is used". Moreover, the need to include test method system suitability data in regulatory submissions has been clearly communicated by regulatory authorities.

USP adopted the following perspectives:

- USP is looking to advance the development and implementation of screening methods for extractables and leachables. Although extractables and leachables screening methods employed by different testing laboratories may vary somewhat in terms of operational details, the methods all must meet a minimum quality standard that is at least partially established by system suitability testing. In this way, testing laboratories are not standardized by the test methods' operating parameters but rather by the test method's operating capabilities. Standardization of system suitability test mixtures may be beneficial so that all testing laboratories establish that their methods meet minimal quality expectations by similar procedures and with similar compounds. Thus, USP is developing standard mixtures with the intent that they would be used by all laboratories performing extractables and leachables screening.
- The USP recognizes the inherent need for flexibility in the implementation of system suitability testing. It is generally well recognized that the overriding requirement for sensitivity in an extractables and leachables screening method is that the method's limit of quantification be less than or equal to the AET. However, the AET is not a constant but rather varies application to application. USP acknowledges and embraces the logic that the absolute and relative concentration of the compounds in the system suitability mixture may vary, application to application and laboratory to laboratory. Therefore, the USP will specify the constituent compounds in the system suitability test mixture, provide a procedure and associated stability data for a mixture that is widely applicable, and speak to an individual laboratory's ability to alter the mixture's concentration and to the individual laboratory's responsibilities when they do so.
- Considering the form of the USP reference standard mixture, two options were considered: 1) providing a premixed mixture of fixed composition; or 2) providing individual reference standard materials and instructions for preparing a mixture at time of use. Considering the aspects of easy-to-use versus flexibility and the issues with potential incompatibilities and stability, the option of providing individual reference compounds and instructions for producing the mixtures would be preferable.
- Individual extractables and leachables screening methods target a specific set of compounds based primarily on volatility. Thus, individual mixtures are proposed for the commonly employed chromatographic screening methods, including:
    - Gas chromatography/mass spectrometry (GC/MS)
    - Headspace-gas chromatography/mass spectrometry (HS-GC/MS)
    - Liquid chromatography/mass spectrometry-atmospheric pressure chemical ionization (LC/MS-APCI)
    - Liquid chromatography/mass spectrometry-electrospray ionization (LC/MS-ESI)
- The optimal mixtures should embrace the concept of efficient and effective system suitability assessment, meaning a system suitability mixture that consists of the minimum number of reference compounds and a system suitability process that includes the minimum number of suitability parameters, assessed with the minimum number of injections.
- Establishing system suitability is a multiple injection process. For example, although sensitivity can be established with a single injection, precision and linearity require multiple injections. Additionally, response stability requires multiple injections be made throughout the entire analytical run.

2. EXPERIMENTAL

2.1 Reagents and Standards

Diluents:

- Methanol, suitable for residue analysis or equivalent
- Dichloromethane, suitable for residue analysis or equivalent
- Ultra-pure water, Type 1

Individual reference standards:

- Analytical grade reagents (purity 98% or greater) for the system suitability compounds and internal standards listed in *Table 3*, *Table 4*, *Table 5*, and *Table 6*

Auxiliary reagents:

- Anhydrous Na_2SO_4, analytical grade quality (ACS or equivalent)

2.2 Laboratory Items

- Analytical balance, minimal readability of 0.1 mg
- 20-mL glass headspace vials with aluminum crimp-caps and Teflon-lined silicone septa
- 2-mL glass autosampler vials with aluminum crimp-caps or plastic screwcaps and Teflon-lined silicone septa
- Class A volumetric glassware
- Gastight syringes of different volumes
- Automatic pipettes of different volumes
- Manual or electronic crimpers for headspace vials

2.3 Preparation of the System Suitability Standard Mixture (Recipe)

- **HS-GC/MS:**

  Single-compound stock solutions containing the following compounds in the following amounts were prepared in methanol, taking care to ensure complete transfer:

  - Acetone-D_6, acetone, dimethoxymethane, cyclohexanone: 2000–10,000 mg/L
  - Toluene-D_8, ethyl acetate, toluene, 4-bromofluorobenzene: 1000–10,000 mg/L
  - Ethanol and other target compounds: 30,000–50,000 mg/L

A multicompound *System suitability stock solution* was prepared by appropriate dilution of the single-compound standard solutions with methanol to achieve the following concentrations:

- Ethanol: 6500 ± 650 mg/L
- Acetone-D_6: 260 ± 26 mg/L
- Acetone: 325 ± 32.5 mg/L
- Dimethoxymethane: 325 ± 32.5 mg/L
- Ethyl acetate: 32.5 ± 3.25 mg/L
- Toluene-D_8: 40 ± 2 mg/L
- Toluene: 3.25 ± 0.33 mg/L
- Cyclohexanone: 650 ± 65 mg/L
- 4-Bromofluorobenzene: 65 ± 6.5 mg/L
- *n*-Tetradecane: 32.5 ± 3.25 mg/L

At time of use, the System suitability test sample was prepared by adding 20 µL of the *System suitability solution* to a 20-mL headspace vial containing 4.0 ± 0.4 g of anhydrous Na_2SO_4 and 13 mL of dilution water resulting in the analyte concentrations stated in *Table 3*.

- **GC/MS:**

  Single-compound stock solutions containing cyclohexanone, 2-ethyl-1-hexanol, 2-ethylhexanoic acid, epsilon-caprolactam, butylated hydroxytoluene (BHT), *n*-nonadecane, 2-heptadecanone, tri-*n*-pentyl phosphate, pyrene, bis(2-ethylhexyl) phthalate (DEHP), *n*-heptacosane, Irgafos 168, and 18-pentatria-contanone were prepared in the concentration range of 1000–5000 mg/L in dichloromethane.

  An *Internal standard solution* (ISS) was prepared in methanol containing 2-fluorobiphenyl (1000 ± 25 mg/L), caffeine-(trimethyl-$^{13}C_3$), and Tinuvin 327 (each 200 ± 20 mg/L).

  A multi-compound *System suitability solution* was prepared by appropriate dilution of the single-compound standard solutions with dichloromethane, resulting in the concentrations stated in *Table 4*. At time of use, the System suitability test sample was prepared by adding 1.0 mL of the *System suitability solution* and 10 µL of the *Internal standard solution* to a 2-mL autosampler vial resulting in the concentrations stated in *Table 4*.

- **LC/MS-APCI:**

  Single-compound stock solutions of epsilon-caprolactam, *N*,*N*-diethylcyclohexylamine, propyl paraben, BHT, Hostanox 03, erucamide, Irgafos 168, and Irganox PS802 were prepared in the concentration range of 1000–5000 mg/L in dichloromethane. Single-compound stock solutions of 4-aminobenzoic acid, 2-mercaptobenzothiazole, palmitic acid, DEHP, and trimyristin were prepared in the concentration range of 1000–5000 mg/L in methanol.

  An ISS was prepared in methanol containing 2-fluorobiphenyl (1000 ± 25 mg/L), caffeine-(trimethyl-$^{13}C_3$), and Tinuvin 327 (each 200 ± 20 mg/L).

  A multi-compound *System suitability solution* was prepared by appropriate dilution of the single-compound standard solutions with methanol resulting in the concentrations stated in *Table 5*.

  At time of use, the System suitability test sample was prepared by adding 1.0 mL of the *System suitability solution* (as described above) and 10 µL of the *Internal standard solution* to a 2-mL autosampler vial resulting in the concentrations stated in *Table 5* for the system suitability compounds and concentrations of 2.0 mg/L for the internal standards caffeine-(trimethyl-$^{13}C_3$) and Tinuvin 327.

- **LC/MS-ESI:**

  Single-compound stock solutions consisting of L-phenylalanine-^{15}N, *N*,*N*-diethylcyclohexylamine, perfluorooctanoic acid (PFOA), 4-aminobenzoic acid, adipic acid, epsilon-caprolactam, propyl paraben, 2-mercaptobenzothiazole, erucamide, and Irganox 1076 were prepared in the concentration range of 2000 to 5000 mg/L in methanol. A stock solution of citric acid was prepared in water; a stock solution of guanine was prepared in 1 M hydrochloric acid in water.

  An ISS was prepared in methanol containing caffeine-(trimethyl-$^{13}C_3$), terephthalic acid-2,3,5,6-d_4, calcium d-pantothenate, and bis(2-ethylhexyl) phthalate-3,4,5,6-d_4 (each 100 ± 10 mg/L).

  A multi-compound *System suitability solution* was prepared by appropriate dilution of the single-compound standard solutions with methanol and transferring 1.0 mL of this diluted solution to a 2-mL autosampler vial and adding 10 µL of the *Internal standard solution*

results in the final concentrations stated in *Table 6*.

2.4 Chromatographic Methods Used to Generate the Example Chromatograms

- **HS-GC/MS:**

Instrument: Agilent 7697A headspace sampler and Agilent 6890N, 7890B or 8890 Gas Chromatography System/Agilent 5975, 5975B, 5977A or 5977B Single Quad Mass Spectrometer

Column: DB-624 60-m × 0.25-mm × 1.4-µm (Agilent part number 122-1364)

Instrument operating parameters

Headspace parameters

Vial temperature: 75°

Loop volume: 1 mL

Vial equilibration time: 20 min

GC parameters

Carrier gas and flow rate: Helium in constant flow rate mode, retention time (t_R) locked to toluene-D_8 at 17.0 min

Injection mode: Split, split ratio 10:1

Inlet temperature: 220°

Oven program: 3 min at 45°, to 90° (4 min) at 8°/min, to 200° (3 min) at 5°/min, to 220° (5.37 min) at 10°/min

MS parameters

Ionization mode: Electron impact

Acquisition mode: Full scan

Mass range (*m/z*): 35–300

- **GC/MS:**

Instrument: Agilent 7683B, 7693, or 7693A Auto Sampler/Agilent 6890N, 7890A, 7890B or 8890 Gas Chromatography System/Agilent 5975B, 5975C, or 5977B Single Quad Mass Spectrometer

Column: HP-5MS Ultra Inert 30-m × 0.25-mm × 0.25-µm (Agilent part number 19091S-433UI)

Instrument operating parameters

GC parameters

Carrier gas and flow rate: Helium in constant flow rate mode, t_R locked to 2-fluorobiphenyl at 16.8 min

Injection mode: Splitless, splitless time 1.25 min

Inlet temperature: 270°

Injection volume: 1 µL

Oven program: 4 min at 50°, to 300° at 8°/min, 12 min at 300°

MS parameters

Ionization mode: Electron impact

Acquisition mode: Full scan

Mass range (*m/z*): 35–700

- **LC/MS-APCI:**

Instrument: Thermo Vanquish UPLC/Exploris 120 Orbitrap Mass Spectrometer

Column: Waters Acquity CSH-C18 100-mm × 3.0-mm × 1.7-µm (Waters part number 186005301)

Instrument operating parameters

LC gradient: See *Table 1*.

Flow rate: See *Table 1*.

Column temperature: 40°

Injection volume: 5 µL

MS parameters

Acquisition mode: Full scan APCI+/APCI−

Mass range (*m*/*z*): 100–1500

Table 1. Mobile Phase Gradient, LC/MS-APCI

Time (min)	Flow Rate (µL/min)	UPW[a] (%)	Methanol (%)
0	500	80	20
7.0	500	0	100
25.0	500	0	100
26.0	500	80	20
29.0	500	80	20

[a] UPW, ultrapure water.

- **LC/MS-ESI:**

  Instrument: Thermo Scientific UltiMate 3000 RS Auto Sampler/UltiMate 3000 RS Pump/UltiMate 3000 RS Diode Array Detector/Q-Exactive Focus Orbitrap Mass Spectrometer

  Column: Waters Acquity HSS-C18 100-mm × 2.1-mm × 1.8-µm (Waters Part number 186003533)

  Instrument Operating Parameters

  LC gradient: See *Table 2*.

  Flow rate: See *Table 2*.

  Column temperature: 40°

  Injection volume: 5 µL

  MS Parameters

  Acquisition mode: Full scan ESI+/ESI−

  Mass range (*m*/*z*): 100–1500

Table 2. Mobile Phase Gradient, LC/MS-ESI

Time (min)	Flow Rate (µL/min)	Methanol + 0.05% Formic acid (%)	UPW[a] + 0.05% Formic acid (%)
0	250	2	98
15.5	250	65	35
17.5	250	98	2
18.0	250	100	0
19.5	500	100	0
29.5	500	100	0
32.0	250	2	98
37.0	250	2	98

[a] UPW, ultrapure water.

3. RESULTS AND DISCUSSION

3.1 Standard Mixture for HS-GC/MS

To view the composition of the standard mixture, see *Table 3*.

Table 3. Composition of the HS-GC/MS Suitability Mixture. Solvent = Ultrapure water

Compound	CAS Number	Peak Number	Retention Time (min)	Concentration (mg/L)
Ethanol	1-17-5	1	6.96	10 (1012768)[a]
Acetone-D_6[b]	666-52-4	2	7.59	0.4
Acetone	67-64-1	3	7.71	0.5 (1006801)
Dimethoxymethane	109-87-5	4	7.71	0.5
Ethyl acetate	141-78-6	5	10.60	0.05 (1265300)
Toluene-D_8[c]	2037-26-5	6	16.99	0.06
Toluene	108-88-3	7	17.14	0.005 (1601805)
Cyclohexanone	108-94-1	8	24.38	1.0
4-Bromofluorobenzene	460-00-4	9	24.58	0.10
n-Tetradecane	629-59-4	10	39.39	0.05

[a] The numbers in parentheses refer to the USP catalog number for existing reference standards.
[b] Secondary internal standard.
[c] Primary internal standard.

3.1.1 Justification of the Composition of the Standard Mixture

The composition of the standard mixture is justified by assigning its individual members to specific functions as follows:

- **Anchor compounds:** Ethanol and *n*-tetradecane
- **Sensitivity compounds:** Toluene (primary), ethyl acetate (secondary)
- **Critical pair:** Cyclohexanone and 4-bromofluorobenzene (primary), toluene and toluene-D8 (secondary)
- **Overlapping compound:** Cyclohexanone (with GC/MS)
- **Special purpose compounds**
  - **Data processing check, deconvolution:** Dimethoxymethane and acetone are close-eluting compounds that have multiple common ions and are used for testing the data processing system's peak deconvolution capabilities.
  - **Data processing check, identification by spectral matching:** 4-Bromofluorobenzene
  - **Potential toxic substances:** None
- **Compounds present to add chemical diversity:** Acetone, dimethoxymethane
- **Precision:** Ethyl acetate, cyclohexanone, *n*-tetradecane

Each member of the standard mixture serves as a marker for one or more relevant functions. All relevant functions are covered by one or more members of the standard mixture. Although there is some redundancy in the mixture in terms of compounds and functions, it is not excessive. The compounds in the reference mixture represent differing functionalities and chemical properties, limited to the extent that they be sufficiently volatile to be amenable to HS-GC/MS analysis.

3.1.2 Discussion of the Standard Mixture's Chromatographic Performance

As is illustrated in *Figure 3*, the reference compounds elute evenly distributed throughout the entire chromatogram, with a modest bias towards early eluting substances. The chromatographic peaks are generally narrow, symmetrical, and well resolved, except in the case of critical pairs.

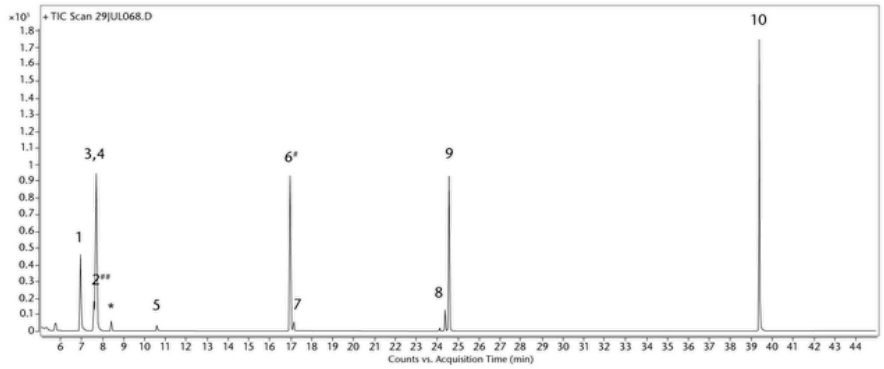

Figure 3. Typical chromatogram, HS-GC/MS system suitability mixture. See *Table 3* for peak numbering. *, system peak; #, internal standard peak (toluene-D_8); ##, secondary internal standard peak (acetone-D_6).

3.2 Standard Mixture for "Direct Injection" GC/MS

To view the composition of GC/MS standard mixture, please see *Table 4*.

Table 4. Composition of the GC/MS Suitability Mixture. Solvent = Dichloromethane (DCM)

Compound	CAS Number	Peak Number	Retention Time (min)	Concentration (mg/L)
Cyclohexanone	108-94-1	1	7.02	20
2-Ethyl-1-hexanol	104-76-7	2	10.21	10
2-Ethylhexanoic acid	149-57-5	3	12.44	50
ε-Caprolactam	105-60-2	4	14.75	20 (1091039)[b]
2-Fluorobiphenyl (internal standard)[a]	321-60-8	5	16.77	10
Butylated hydroxytoluene, BHT	128-37-0	6	19.09	1 (1082708)[b]
Caffeine-(trimethyl-$^{13}C_3$)	78072-66-9	7	23.80	2
n-Nonadecane	629-92-5	8	24.34	5
2-Heptadecanone	2922-51-2	9	24.39	5
Tri-*n*-pentyl phosphate	2528-38-3	10	24.73	5
Pyrene	129-00-0	11	27.17	1
Bis(2-ethylhexyl) phthal-ate, DEHP	117-81-7	12	31.50	1 (1545056)
2,4-Di-tert-butyl-6-(5-chloro-2*H*-benzotriazol-2-yl) phenol (Tinuvin 327)	3864-99-1	13	32.71	2
n-Heptacosane	593-49-7	14	32.83	1

Compound	CAS Number	Peak Number	Retention Time (min)	Concentration (mg/L)
Tris(2,4-di-tert-butylphenyl) phosphite (Irgafos 168)	31570-04-4	15	40.63	10 (1544964)
18-Pentatriacontanone	504-53-0	16	45.77	50

[a] 2-Fluorobiphenyl is the primary internal standard used with this method. Caffeine-(trimethyl-$^{13}C_3$) and Tinuvin 327 can be used as optional secondary internal standards.

[b] The numbers in parentheses refer to the USP catalog number for existing reference standards.

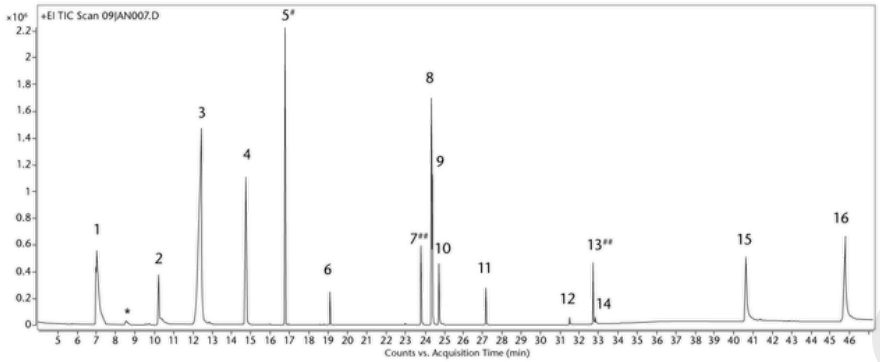

Figure 4. Typical chromatogram, GC/MS system suitability mixture. See *Table 4* for peak labeling. *, system peak; #, internal standard peak (2-fluorobiphenyl); ##, secondary internal standard peaks (caffeine-(trimethyl-$^{13}C_3$) and Tinuvin 327).

3.2.1 Justification of the Composition of the Standard Mixture

The composition of the standard mixture is justified by assigning its individual members to specific functions as follows:

- **Anchor compounds:** Cyclohexanone and 18-pentatriacontanone
- **Sensitivity compounds:** BHT (strong responder), 2-ethyl-1-hexanol and *n*-heptacosane (poor responders)
- **Critical pair:** Tinuvin 327 and *n*-heptacosane (primary), *n*-nonadecane and 2-heptadecanone (secondary)
- **Overlapping compound:** Cyclohexanone (with HS-GC/MS); BHT, DEHP, Tinuvin 327, and Irgafos 168 (with LC/MS-APCI); ε-caprolactam with LC/MS-APCI and LC/MS-ESI
- **Special purpose compounds**
  - **Data processing check, deconvolution:** 2-Heptadecanone and *n*-nonadecane are close-eluting compounds having multiple common ions and are used for testing the data processing system's peak deconvolution capabilities.
  - **Data processing check, identification by spectral matching:** Tri-*n*-pentyl phosphate
  - **Potential toxic substances:** 2-Ethyl-1-hexanol, 2-ethylhexanoic acid, BHT, tri-*n*-pentyl phosphate, pyrene, DEHP, Tinuvin 327
- **Compounds present to add chemical diversity:** 2-Ethylhexanoic acid
- **Precision:** 2-Ethyl-1-hexanol, *n*-nonadecane, Irgafos 168

Each member of the standard mixture serves as a marker for one or more relevant functions. All relevant functions are covered by one or more members of the standard mixture. Although there is some redundancy in the mixture in terms of compounds and functions, it is not excessive. The compounds in the reference mixture represent differing functionalities and chemical properties, limited to the extent that they be sufficiently semivolatile to be amenable to GC/MS analysis.

3.2.2 Discussion of the Standard Mixture's Chromatographic Performance

As is illustrated in *Figure 4*, the reference compounds elute throughout the entire chromatogram. The chromatographic peaks are generally narrow, symmetrical, and well-resolved, except in the case of critical pairs.

3.3 Standard Mixture for LC/MS-APCI

To view the composition of the standard mixture, see *Table 5*.

Table 5. Composition of the LC/MS-APCI Suitability Mixture. Solvent = Methanol (MeOH)

Compound	CAS Number	Peak Number	Retention Time (min)	Mode	Concentration (mg/L)
4-Aminobenzoic acid	150-13-0	1	1.30	±	10
ε-Caprolactam	105-60-2	2	2.49	+	1 (1091039)[a]
2-Mercaptobenzothiazole	149-30-4	3	5.05	±	1
N,*N*-Diethylcyclohexylamine	91-65-6	4	5.88	+	10
Propyl paraben	94-13-3	5	5.88	±	1 (1577008)
Butylated hydroxy-toluene (BHT)	128-37-0	6	8.32	–	1 (1082708)
Ethane-1,2-diyl bis(3,3-bis(3-(tert-butyl)-4-hydroxyphenyl) butanoate) (Hostanox 03)	32509-66-3	7	8.40	–	1 (1544927)
Palmitic acid	57-10-3	8	9.33	–	1 (1492007)
Bis(2-ethylhexyl) phthalate (DEHP)	117-81-7	9	9.41	+	1 (1545056)
Erucamide	112-84-5	10	9.95	±	1 (1545045)
2,4-Di-tert-butyl-6-(5-chloro-2*H*-benzotriazol-2-yl) phenol (Tinuvin 327) (internal standard)	3864-99-1	11	10.94	±	1
Tris(2,4-di-tert-butylphenyl) phosphite (Irgafos 168)	31570-04-4	12	15.35	±	1 (1544964)
Trimyristin	555-45-3	13	24.71	+	5
Dioctadecyl 3,3′-sulfanediyldipropanoate (Irganox PS802)	693-36-7	14	27.56	+	1 (15445012)

[a] The numbers in parentheses refer to the USP catalog number for existing reference standards.

See *Figure 5* and *Figure 6* for example chromatograms.

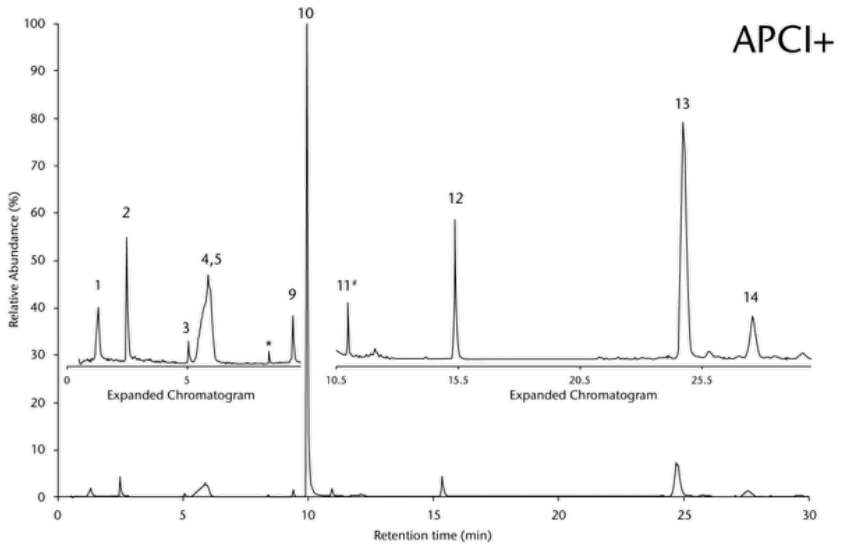

Figure 5. Typical chromatogram, LC/MS-APCI+ system suitability mixture. See *Table 5* for peak labeling. *, system peak; #, internal standard peak (Tinuvin 327). Note that the actual chromatographic run ends at 29 min, followed by column rinsing and re-equilibration.

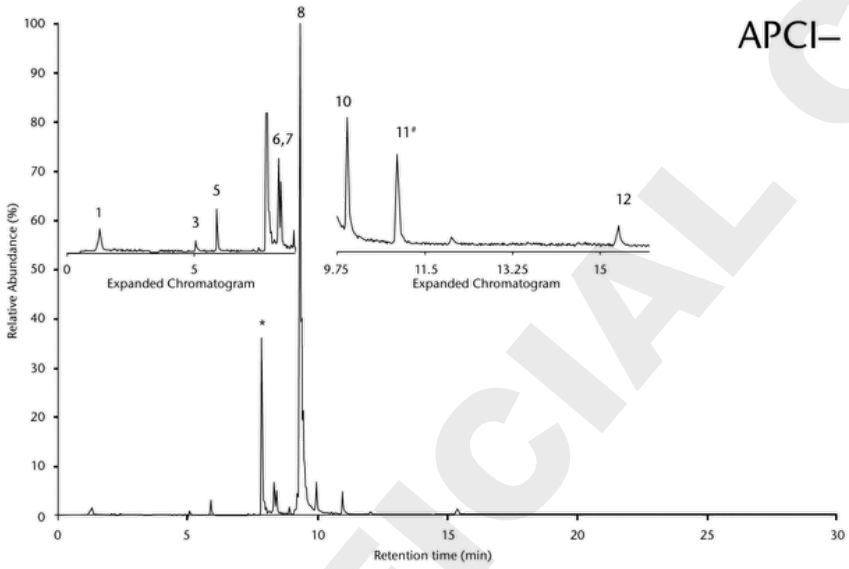

Figure 6. Typical chromatogram, LC/MS-APCI– system suitability mixture. See *Table 5* for peak labeling. *, system peak; #, internal standard peak (Tinuvin 327). Note that the actual chromatographic run ends at 29 min, followed by column rinsing and re-equilibration.

3.3.1 Justification of the composition of the standard mixture

The composition of the standard mixture is justified by assigning its individual members to specific functions as follows:

APCI+:

- **Anchor compounds:** 4-Aminobenzoic acid and Irganox PS802
- **Sensitivity compounds:** 4-Aminobenzoic acid and DEHP
- **Critical pair:** BHT and Hostanox 03
- **Overlapping compounds:** 4-Aminobenzoic acid, 2-mercaptobenzothiazole, propyl paraben, erucamide, Tinuvin 327, and Irgafos 168 overlap with LC/MS-APCI-; 4-aminobenzoic acid, *N,N*-diethylcyclohexylamine, propyl paraben, and erucamide overlap with LC/MS-ESI+; epsilon-caprolactam, DEHP, Tinuvin 327, and Irgafos 168 overlap with GC/MS
- **Special purpose compounds**
- - **Data processing check, deconvolution:** Propyl paraben has a good peak shape and co-elutes with the broad peak of *N,N*-diethylcyclohexylamine

- **Data processing check, spectral matching:** Not applicable
- **Potential toxic substances:** 4-Aminobenzoic acid, 2-mercaptobenzothiazole, *N,N*-diethylcyclohexyl-amine, propyl paraben, DEHP, Tinuvin 327

- **Compounds present to add chemical diversity:** Trimyristin
- **Precision:** 2-Mercaptobenzothiazole, erucamide, Irgafos 168

APCI–:

- **Anchor compounds:** 4-Aminobenzoic acid and Irgafos 168
- **Sensitivity compounds:** 4-Aminobenzoic acid and 2-mercaptobenzothiazole
- **Critical pair:** BHT and Hostanox 03
- **Overlapping compound:** 4-Aminobenzoic acid, 2-mercaptobenzothiazole, propyl paraben, erucamide, Tinuvin 327, and Irgafos 168 overlap with LC/MS-APCI+; 4-aminobenzoic acid, propyl paraben, and erucamide overlap with LC/MS-ESI–; BHT, Tinuvin 327, and Irgafos 168 overlap with GC/MS.
- **Special purpose compounds**
- - Data processing check, deconvolution: Not applicable
  - Data processing check, spectral matching: Not applicable
  - Potential toxic substances: 4-Aminobenzoic acid, 2-mercaptobenzothiazole, propyl paraben, BHT, Hostanox 03, Tinuvin 327
- **Compounds present to add chemical diversity:** Palmitic acid
- **Precision:** 2-Mercaptobenzothiazole, erucamide, Irgafos 168

Each member of the standard mixture performs one or more relevant functions. All relevant functions are covered by one or more members of the standard mixture. Although there is some redundancy in the mixture in terms of compounds and functions, it is not excessive. The compounds in the reference mixture represent differing functionalities and chemical properties, limited to the extent that they be sufficiently non-volatile and non-polar to be amenable to LC/MS and detectable by APCI analysis.

3.3.2 Discussion of the Standard Mixture's Chromatographic Performance

APCI+:

As is illustrated in *Figure 5*, the reference compounds elute throughout the entire chromatogram. The chromatographic peaks are generally narrow, symmetrical, and well resolved, except in the case of critical pairs.

APCI–:

As is illustrated in *Figure 6*, the reference compounds elute primarily in the early to middle parts of the chromatogram and the latter part of the chromatogram is poorly represented. The chromatographic peaks are generally narrow, symmetrical, and well resolved, except in the case of critical pairs.

3.4 Standard Mixture for LC/MS-ESI

Table 6. Composition of the LC/MS-ESI Suitability Mixture. Solvent = UPW/MeOH 98/2, v/v

Compound	CAS Number	Peak Number	Retention Time (min)	Mode	Concentration (mg/L)
Guanine	73-40-5	1	2.04	±	1 (1302156)[a]
Citric acid	77-92-9	2	2.35	–	10 (1134368)
L-Phenylalanine-15N (secondary internal standard, + ion)	29700-34-3	3	4.87	±	1
4-Aminobenzoic acid	150-13-0	4	5.46	+	1
N,N-Diethylcyclohexylamine	91-65-6	5	6.55	+	1
Adipic acid	124-04-9	6	6.60	–	1 (1012190)
ε-Caprolactam	105-60-2	7	7.35	+	1 (1091039)

Compound	CAS Number	Peak Number	Retention Time (min)	Mode	Concentration (mg/L)
Caffeine-(trimethyl-$^{13}C_3$) (primary internal standard, + ion)	78072-66-9	8	8.80	+	1
Terephthalic-D4 acid (internal standard, - ion)	60088-54-2	9	9.63	–	1
2-Mercaptobenzothiazole	149-30-4	10	14.04	±	1
Propyl paraben	94-13-3	11	16.75	±	1 (1577008)
Perfluorooctanoic acid	335-67-1	12	18.85	–	0.2
Erucamide	112-84-5	13	20.08	+	1 (1545045)
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (Irganox 1076)	2082-79-3	14	22.59	+	1 (1544950)

[a] The numbers in parentheses refer to the USP catalog number for existing reference standards.

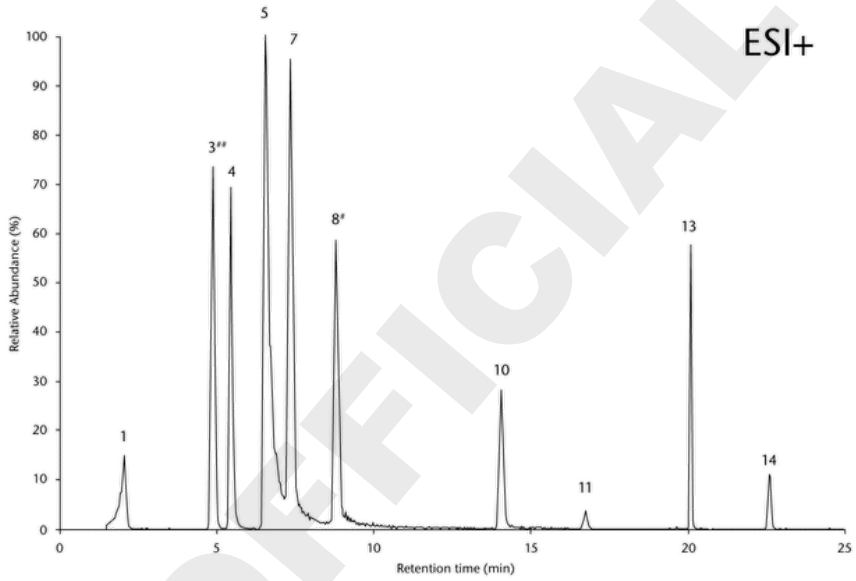

Figure 7. Typical chromatogram, LC/MS-ESI+ system suitability mixture. Refer to *Table 6* for peak numbers. #, internal standard peak (caffeine-(trimethyl-13C3)); ##, secondary internal standard 1 peak (L-phenylalanine-^{15}N). Note that the actual chromatographic run ends at 29.5 min, followed by re-equilibration.

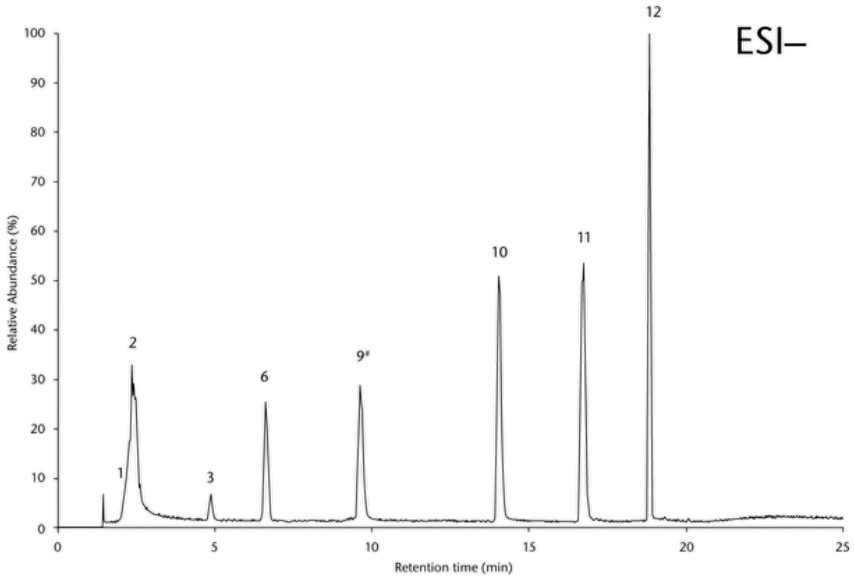

Figure 8. Typical chromatogram, LC/MS-ESI– system suitability mixture. Refer to *Table 6* for peak numbers. #, internal standard peak (terephthalic-D_4 acid). Note that the actual chromatographic run ends at 29.5 min, followed by re-equilibration.

3.4.1 JUSTIFICATION OF THE COMPOSITION OF THE STANDARD MIXTURE

The composition of the standard mixture is justified by assigning its individual members to specific functions as follows:

ESI+:

- **Anchor compounds:** Guanine and erucamide
- **Sensitivity compounds:** Propyl paraben
- **Critical pair:** L-Phenylalanine-^{15}N and 4-aminobenzoic acid
- **Overlapping compound:** Propyl paraben overlaps with ESI-; 4-aminobenzoic acid, *N,N*-diethylcyclohexylamine, ε-caprolactam, 2-mercaptobenzothiazole, propyl paraben, and erucamide overlap with LC/MS-APCI+; ε-caprolactam overlaps with GC/MS
- **Special purpose compounds**
  - Data processing check, deconvolution: Not applicable
  - Data processing check: Not applicable
  - Potential toxic substances: 4-Aminobenzoic acid, 2-mercaptobenzothiazole, *N,N*-diethylcyclohexylamine, propyl paraben
- **Compounds present to add chemical diversity:** Guanine
- **Precision:** 4-Aminobenzoic acid, 2-mercaptobenzothiazole, Irganox 1076

ESI–:

- **Anchor compounds:** Citric acid and Irganox 1076
- **Sensitivity compounds:** Perfluorooctanoic acid
- **Critical pair:** Guanine and citric acid
- **Overlapping compound:** Propyl paraben overlaps with ESI+; 4-aminobenzoic acid, *N,N*-diethylcyclohexylamine, 2-mercaptobenzothiazole, propyl paraben, and erucamide overlap with LC/MS-APCI–; note that there are no compounds that overlap with GC/MS
- **Special purpose compounds**
  - Data processing check: Not applicable
  - Data processing check: Not applicable
  - Potential toxic substances: 2-Mercaptobenzothiazole, propyl paraben, perfluorooctanoic acid
- **Compounds present to add chemical diversity:** Guanine, adipic acid, perfluorooctanoic acid
- **Precision:** Adipic acid, 2-mercaptobenzothiazole, Irganox 1076

3.4.2 DISCUSSION OF THE STANDARD MIXTURE'S CHROMATOGRAPHIC PERFORMANCE

ESI+:

As is illustrated in *Figure 7*, the reference compounds elute evenly throughout the entire chromatogram. The chromatographic peaks are generally narrow, symmetrical, and well resolved, except in the case of critical pairs.

ESI-:

As is illustrated in *Figure 8*, the reference compounds elute primarily in the early to middle parts of the chromatogram and the latter part of the chromatogram is under-represented. The chromatographic peaks are generally narrow, symmetrical, and well resolved, except in the case of critical pairs.

3.5 System Suitability Acceptance Criteria

A screening method for organic extractables is considered a success if it meets three criteria:

1. The method can detect (i.e., produces a recognizable response for) all potential extractables above the AET.
2. The method produces data that allows one to correctly identify all detected extractables above the AET.
3. The method produces data that allows one to accurately quantify all identified extractables above the AET.

Thus, it is logical that system suitability testing applied to a chromatographic screening method adopts these same expectations as its essential acceptance criteria. Therefore, the essential acceptance criteria applied to system suitability testing using USP system suitability mixtures are as follows:

- The screening method being evaluated must produce a recognizable response for all compounds present in the mixture
- Processing of the data produced by the screening method must lead to the proper identification of all the compounds present in the mixture
- Processing of the data produced by the screening method must produce accurate concentrations for all the compounds present in the mixture (meaning that the calculated value for the compound's concentration is within 50% to 200% of its prepared concentration)

The term "produce a recognizable response" is somewhat subjective as the screening methods for organic extractables cannot always easily follow traditional means for establishing detectability. For example, the concentration of an analyte that produces a peak whose size is equal to 3 times the peak-to-peak noise is generally recognized as the limit of detection (LoD). However, as it can be difficult to estimate the peak-to-peak noise in chromatographic screening methods used for extractables, this means of determining the LoD is not always useful in extractables testing. Thus, it is left up to the individual testing lab to establish, and justify, the means by which it has determined that a compound in the standard mixture has produced a recognizable signal. Nonetheless, it is noted that because the compound must also be identified and quantified to meet the acceptance criteria, the concept of a recognizable response should include the requirement that the response has sufficient information to produce the correct identity and an accurate quantitation.

The acceptance criteria for precision requires additional discussion as precision is established based on response variation for multiple injections. For injection-to-injection precision, the determination involves multiple injections of the system suitability mixture at the beginning of the chromatographic run. For inter-run precision, these injections at the beginning of the run are supplemented by injections made throughout the run.

For injection-to-injection precision, it is envisioned that in typical operation, a chromatographic system is "conditioned" by replicate injection of a conditioning solution (which could be the system suitability mixture) until a constant response is obtained. Once a system has been properly conditioned, system suitability is addressed by making three additional injections of the system suitability mixture. The system suitability parameters noted earlier (detect each peak, properly identify each peak, and confirm concentration) are typically performed on the last of these three injections. Injection-to-injection precision is calculated based on the responses obtained for all three precision-indicating compounds in all three injections and is typically represented by the percent relative standard deviation (%RSD) of the responses. A reasonable expectation for injection-to-injection precision in trace analysis is a %RSD of not more than (NMT) 15% for each of the three compounds specified as precision markers.

Alternatively, inter-run precision is addressed by injecting the system suitability mixture throughout the chromatographic run (e.g., every 15 injections). In this case, the system suitability measure becomes the %RSD calculated for all injections of the system suitability mixture made throughout the chromatographic run. As it is reasonable to expect that inter-run imprecision would be somewhat greater than injection-to-injection imprecision, a reasonable acceptance criterion for inter-run precision is %RSD NMT 20%.

In certain cases, a portion of an analytical run might meet the acceptance criterion for inter-run precision even though the entire runs fail to meet the criterion, resulting in the run being rejected. It is an acceptable practice to try and salvage that portion of the run where the response was acceptably stable. In such circumstances, the portion of the run that is salvageable is that portion of the run that is bracketed by the start of the run and the last injection of the system suitability mixture that met the %RSD requirement.

It is possible that with further experience and after further consideration, other acceptance criteria could be developed for the system suitability mixtures. For example, it might be possible that the acceptance criteria could include a value for a minimum resolution between the critical pair. Currently, however, development of such secondary acceptance criteria remains a "next step" activity.

4. CONCLUSIONS

A practical rationale for using standardized reference standard mixtures to support system suitability assessment of chromatographic screening methods used for extractables and leachables has been proposed. Potential USP reference standard mixtures have been developed for the following analytical methods based on this practical rationale:

- Headspace GC/MS, generally applied for volatile compounds
- "Direct injection" GC/MS, generally applied for semi-volatile compounds
- "Direct injection" LC/MS, generally applied for non-volatile compounds including both APCI and ESI ionization

Rather than providing the reference standard mixtures in a ready-to-use form, USP likely will provide individual reference compounds and the sample preparation instructions for the generation of such mixtures.

USP is looking for feedback from extractables and leachables stakeholders about the appropriateness and rationale indicated in this *Stimuli* article. Based on the collected feedback, USP will make necessary and appropriate adjustments to the proposed reference standard mixtures.

5. NEXT STEPS

5.1 Collection of Feedback from Stakeholders

The proposal of new standard approaches is typically preceded by an extensive period of stakeholder engagement designed to insure that a) the product addresses a stakeholder need, and b) the developed product fills that need. Thus, as USP develops new reference standard mixtures for extractables and leachables suitability assessments, it is appropriate that USP would engage stakeholders to determine the appropriateness of the proposed mixtures. In the context of reference standard solutions for system suitability testing, this means:

1. Appropriateness of the proposal:
    A. Do stakeholders foresee the need for, and the value in having, reference standards solutions to facilitate system suitability testing in the chromatographic screening methods applied to extractables and leachables?
    B. USP encourages stakeholders to provide their opinions as to the USP approach to providing reference standards and reference standard mixtures.
2. Appropriateness of the proposed mixtures:
    A. USP encourages stakeholders to share their opinions in terms of the specific system suitability mixtures proposed in this *Stimuli* article considering a) the number of compounds in the mixture(s); b) the identities of the compounds in the mixture(s); c) the concentrations of the compounds in the mixture; d) the solvent in which the mixture is prepared in; and e) the suitability of the reference standard mixtures.
    B. USP encourages stakeholders to provide suggestions as how to improve the system suitability mixtures in terms of their composition.
3. Appropriateness of potential future system suitability mixtures:
    A. USP encourages stakeholders to provide their input about other reference standards and standard mixtures that USP could consider developing.

To obtain this valuable and necessary feedback, the USP will be using multiple avenues to obtain public comments and foster public discussions, including this *Stimuli* article, presentations at conferences, communications via the USP website, and potential USP-sponsored workshop(s).

5.2 Revision of Standard Mixtures (as necessary and appropriate)

Based on stakeholder feedback, the USP will reconsider its proposed system suitability mixtures, focusing specifically on composition and practicality.

Once finalized, system suitability mixtures will be communicated to the extractables and leachables community by appropriate means.

5.3 Standard Availability

The currently proposed system suitability mixtures include several individual reference compounds that are already available as individual USP Reference Standards. Those currently available individual reference standards present in the USP system suitability mixtures were identified in the individual tables listing the mixtures' composition (*Table 3*, *Table 4*, *Table 5*, and *Table 6*). However, the mixtures also contain compounds that are not currently available as individual USP Reference Standards.

Once the system suitability mixtures have been publicly reviewed, revised as necessary and finalized, USP will begin, and complete, the task of qualifying batches of high-quality materials as Reference Standards. At some time in the future, if necessary and possible, USP may offer these standard mixtures as a ready-to-use prepared solution after evaluating their stability and compatibility.

5.4 Stability of the System Suitability Mixtures

The longer a reference mixture is stable, the more convenient it is to use, as long shelf-life precludes the necessity to either repetitively prepare or purchase the reference mixture. The USP will address the issue of shelf-life from two perspectives, the shorter-term stability of a test mixture prepared per the USP instructions from the individual USP Reference Standards materials at time of use and the longer term stability of the same test mixture as a prelude to the USP possibly offering a ready-to-use reference standard mixture. The stability assessment will consider not only duration but also temperature of storage, as ambient temperature or refrigerated storage is more convenient than frozen storage. The initial intent of this exercise will be to establish the shelf-life of the USP standard mixtures prepared at time of use, thus providing laboratories with proper storage requirements. Should it be the case that the mixtures are highly stable, such a finding could be the catalyst for USP to consider the development of so-called pre-mixed, ready-to-use standard mixtures.

5.5 The Development of Other Extractables and Leachables Standard Mixtures

Although establishing that a chromatographic screening test method for organic extractables and leachables was set up properly and that the testing equipment is functioning properly is clearly a critical aspect of producing reliable and accurate analytical data, this type of system suitability testing reflects only a small portion of suitability for use assessment necessary to insure that extractables and leachables data is accurate and precise and that optimum analytical sensitivity has been attained. As reference standard mixtures are a critical enabler of suitability for use assessment, USP may explore other options to supply the extractables and leachables community with necessary reference materials and mixtures.

Perhaps the most obvious need for additional extractables and leachables reference mixtures is in system suitability testing for routinely utilized screening methods used for analytes other than "classical" organic compounds. Such methods include inductively coupled plasma

spectroscopy (ICP-OES for detection by optical emission spectroscopy and ICP-MS for detection via mass spectrometry) for elements and ion chromatography (IC) for inorganic acids and low molecular organic acids.

The challenge in producing multielement reference standard mixtures for ICP-based testing is the large number of elements that are relevant as extractables or leachables. A significant development in establishing best practices for elemental screening of drug products was the publication of ICH Q3D, Elemental Impurities (*12*), *Elemental Impurities—Limits* ⟨232⟩, and *Elemental Impurities—Procedures* ⟨233⟩ (*13–14*), which address elemental impurities in drug products, including but not limited to elemental leachables. In Q3D and ⟨232⟩, permissible daily exposure (PDE) limits are established for 24 elements, and therefore these 24 elements are generally recognized as being extractables and leachables targets, regardless of whether the elements are commonly encountered. As testing drug products for elemental impurities has become pervasive within the pharmaceutical industry, commercially available, ready-to-use multielement reference standards exist. However, due to incompatibilities, multiple mixtures are required to account for the 24 elemental targets. Moreover, the 24 target elements do not include well known extractable elements such as aluminum, iron, zinc, silicon, and sulfur. For these reasons, it is arguable whether the existing multielement mixtures are well suited for extractables and leachables screening. Thus, USP may consider the development of multielement reference standard mixtures, specifically for the purpose of system suitability testing in ICP-based analyses for extractables and leachables.

IC methods for the analysis of aqueous samples for common inorganic anions (e.g., fluoride, chloride, nitrate, phosphate, sulfate) or low molecular weight volatile organic acids (e.g., acetate and formate) exist and are occasionally applied to extractables leachables screening. Although these targeted chemicals are unlikely to have an adverse effect on patient safety, they can affect a drug product's chemical properties, most notably pH.

Numerous commercial organizations sell ready-to-use, multichemical reference standards containing the targeted inorganic anions and it is unlikely the USP adds value by developing such a reference standard solution. Although reference standard mixtures containing the weak organic acids are not commercially available, likely due to their volatility and instability, it is doubtful that USP adds much value by providing such a standard mixture for extractables and leachables applications. Thus, it is unlikely USP will develop ionic reference standard mixtures to support IC screening for extractables and leachables, especially for the somewhat limited purpose of system suitability assessment. It is noted that the USP already has single-compound Reference Standards for glacial acetic acid (catalog no. 1005706) and formic acid (catalog no. 1283200).

The use of reference standard mixtures can be expanded to serve a purpose other than system suitability assessment. For example, reference standard mixtures would be useful in:

- Recovery studies, whose purpose is to establish that methods used to prepare extracts or drug products for analysis (e.g., solvent switching followed by evaporative concentration) are quantitative and reproducible
- Quantitation
- Establishing a method's ability to detect potential toxic compounds (e.g., cohorts of concern) at appropriately low levels

Consistent with these purposes, reference standard mixtures that serve these purposes would be different in composition than the system suitability mixtures.

Developing such reference standards comes with considerable challenges. Considering quantitation and recovery studies, there is the substantial issue of customization versus standardization. It is obvious that the most effective reference standard mixture for quantitation or a recovery study contains the specific extractables or leachables that are relevant to a specific extractables or leachables study. For example, a reference standard mixture that is well suited for quantitating hexane extractables from a rubber stopper is likely not the best reference mixture for quantitating leachables in a dilute aqueous drug product stored in a multilayered polyolefin container. Given the diversity in extractables and leachables profiles, it is difficult to imagine that a single reference standard mixture, consisting of a reasonable number of individual compounds, would be applicable and suitable in a majority of testing circumstances. This reality suggests that customized reference mixtures, prepared at time of use from individual reference standards, may be the proper path forward.

Even if such standardized mixtures could be identified, practical issues such as mutual compatibility and shelf-life would complicate the development of reference standard mixtures.

These difficulties notwithstanding, the USP is investigating the possibility of providing reference standard mixtures for the purpose of quantitation and performing recovery studies. To wit and recognizing the enormity and diversity of the universe of extractables and leachables and the difficulty in establishing reference mixtures that are representative of the entire universe and which contain a manageable number of reference substances, USP is considering ways of dividing the universe into smaller and more manageable groups and then developing reference mixtures appropriate for and relevant to each group. As a simple example, pharmaceutical packaging could be differentiated from medical devices, and different mixtures could be developed to match extractables unique to these individual groups.

The intrinsic issue with developing a reference standard to establish a method's ability to respond to potential toxic compounds is the lack of proper documentation of what these toxic compounds are (i.e., a list of toxic compounds). Moreover, once such a list is constructed, it is expected that the issues of mutual compatibility and shelf-life of a mixture containing these toxic compounds would be considerable. These headwinds notwithstanding, the USP is examining the possibility of developing reference standard mixtures whose purpose is to establish that a particular test method can respond to "potential toxic compounds" with the required sensitivity.

Another need that has been identified is to make available individual reference standards for extractables and leachables that may be commonly encountered but that are not commercially available (e.g., rubber oligomers). The USP will be compiling a list of needed reference

standards and then investigating means for procuring enough of the purified materials to meet the industry need. The USP encourages stakeholders to help USP identify these necessary but commercially unavailable compounds.

In most of these situations, success will be facilitated by USP's ability to partner with interested parties in the extractables and leachables community. All such interested parties are encouraged to reach out to the USP to cooperate in advancing the state of the art in extractables and leachables screening.

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DocID: GUID-CB1F72B3-D9B8-41DA-BA33-B55690DE7005_10101_en-US
DOI: https://doi.org/10.31003/USPNF_S203084_10101_01
DOI ref: 0smab