Development and Characterization of an LC–MS Method for Quantitating Aqueous Extractables, including Bisphenol A, 1-Formylpiperidine, and Bis-(pentamethylene)-urea, from Plastic Materials

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Abstract

Polymers utilized in medical or pharmaceutical applications (e.g., manufacturing, packaging, delivery systems, and devices) contain additives and impurities due to their formulation and/or processing. Under conditions of use, these ingredients and other extractables may leach from the polymers and accumulate in pharmaceutical products. In order to establish the amounts of compounds that may be extracted from such systems and devices, sensitive and selective analytical methodologies are required. A liquid chromatographic method with mass spectrometric detection has been developed for the purpose of quantifying targeted extractables, including Bisphenol A (BPA), 1-formylpiperidine (FP), and bis-(pentamethylene)-urea (BU) at low concentrations [300 ng/mL (ppb) or less] in aqueous extracting media. The performance characteristics of the developed method were established by assessing accuracy, response linearity, precision, specificity, and solution stability. The method is suitable for the quantitation of BPA, FP, and BPU in the concentration range of 50 to 300 ng/mL, with quantitation limits of 10 ng/mL or less. Although the method was applied to the quantitation of 2,6-di-tert-butyl-4-methylphenol, unsatisfactory results were obtained due to the poor stability of this compound in the aqueous extraction media.

Introduction

Plastic materials are widely used in medical items, such as solution containers, transfusion sets, transfer tubing, devices, and manufacturing systems. The physiochemical nature of these materials provides medical products with their necessary, desirable performance characteristics. Although an important performance characteristic of plastics used in medical/pharmaceutical applications is chemical inertness, interactions between a plastic material and the pharmaceutical product it contacts are well documented (1,2). One such interaction is leaching, the release of plastic material components to the product, where both the identities of the leached substances and their accumulation levels may affect the material's ultimate compatibility with the product.

In order for plastics to possess the performance characteristics required in pharmaceutical applications, "pure" polymers are fortified with additives that accomplish specific objectives. For example, antioxidants are plastic additives that retard a plastic's oxidative degradation. One such antioxidant, 2,6-di-tert-butyl-4-methylphenol (BHT), is used in a number of polymers, including polyethylene, polypropylene, polycarbonate, polyurethane, polyamide, and polyisoprene.

Use of polymers in pharmaceutical systems and devices presents the possibility that extractables could leach from the system or device into a pharmaceutical product that is then administered to a patient in a clinical situation. Accumulation of leachables in the pharmaceutical product may lead to suitability for use issues for that product. One important suitability for use consideration involves the safety of the pharmaceutical product.

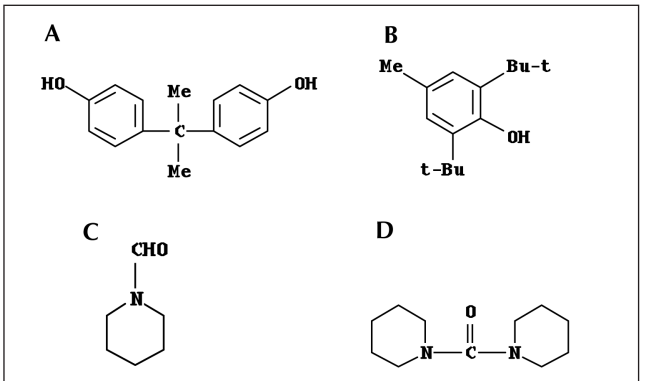

Figure 1. Chemical structures of the analytes of interest.
A = 4,4'-(1-methylethylidene)bis-phenol (Bisphenol A, BPA)
CAS RN 80-05-7, $C_{15}H_{16}O_2$, formula weight = 228.29
B = 2,6-di-(tert-butyl)-4-methyl-phenol (BHT)
CAS RN 128-37-0, $C_{15}H_{24}O$, formula weight = 220.35
C = 1-Formylpiperidine (FP)
CAS RN 2591-86-8, $C_6H_{11}NO$, formula weight = 113.16
D = Bis(pentamethylene)urea (BPU)
CAS RN 5395-04-0, $C_{11}H_{20}N_2O$, formula weight = 196.29

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If the leached substance has undesirable toxicological properties, its accumulation level in the pharmaceutical product must be sufficiently low that its dose to a patient is below the observable effect threshold.

An examination of an elastomeric material for its extractables profile revealed four major extracted substances; Bisphenol A (BPA), 2,6-di-tert-butyl-4-methylphenol (BHT), 1-formylpiperidine (FP), and bis-(pentamethylene)-urea (BPU), see Figure 1. In order to assess potential suitability for use issues associated with a plastic in contact with a pharmaceutical product, extracted or leached substances must be quantified in relevant samples (such as polymer extracts and pharmaceutical products) with a high degree of selectivity and sensitivity. For organic extractables/leachables, chromatographic methods are routinely utilized for this purpose (for example, 2–7 in general, and 8–18 for the analytes of interest). Thus a liquid chromatographic method utilizing mass spectrometric detection (LC–MS) was developed to allow for the quantitation of these extractables in aqueous material extracts at concentrations of 300 ng/mL (ppb) or less. The performance characteristics of the developed method were then determined, consistent with published recommendations for the validation of extractable/leachable assays (19–21).

Experimental

Chemicals and reagents

Solvents and chemicals were obtained commercially in the highest appropriate purity. The individual targeted extractables were obtained from Sigma-Aldrich (St. Louis, MO) and had purities of 99.0% or greater. Methanol [high-performance liquid chromatography (HPLC) grade] was obtained from Honeywell Burdick and Jackson (Morristown, NJ). Ammonium acetate (HPLC grade) was obtained from Aldrich (St. Louis, MO). Distilled laboratory water was used throughout this study.

HPLC System

The chromatographic system was an Applied Biosystems (Foster City, CA) API4000 mass spectrometer coupled to an Agilent (Santa Clara, CA) 1200 HPLC system consisting of a binary pump (G1312A), refrigerated autosampler (G1329A, G1330B), thermostatted column compartment (G1316A), degasser (G1379B), and diode array detector (G1315B). The data was acquired and analyzed via a Dell Precision 390 (Round Rock, TX) Workstation using Applied Biosystems Analyst 1.4.2 software. The chromatographic column was from Waters (Milford, MA), specifically XTerra MS C18 30 × 4.6mm, 2.5 µm particles, PN 186000600.

Chromatographic conditions

The chromatographic conditions used are summarized in Table I. Typical chromatographic performance under these operating conditions is illustrated in Figure 2.

Preparations

Calibration standards were prepared by serial dilution of stock solutions prepared at a nominal concentration of 1000 µg/mL by dissolving the individual reagents in methanol. A composite analyte stock solution, containing 100 µg/mL of each analyte was prepared by dilution of the individual analyte stock solutions with methanol. Intermediate stock solutions were prepared at appropriate concentrations by dilution of the composite stock with methanol. Working calibration standards at target concentrations of 0 (blank), 50, 100, and 300 ppb were prepared by diluting portions of the intermediate stocks with water. A fifth

Table IA. LC–MS Conditions

Parameter	Value		
Column	Waters Xterra MS C18, 30 × 4.6 mm, 2.5 µm		
Column Temperature	~ 40°C		
Injection Volume	10 µL		
Mobile Phase A	10 mM Ammonium Acetate (water)		
Mobile Phase B	Methanol		
	Time (min)	**Flow Rate (mL/min)**	**Proportion B (%)**
Gradient, + ion method	0	0.5	40
	5.0	0.5	75
	8.0	0.5	40
	13.0	0.5	40
Gradient, – ion method	0	0.5	20
	1.0	0.5	20
	4.0	0.5	95
	8.0	0.5	95
	8.2	0.5	20
	13.0	0.5	20
Diverter Valve	**Total Time (min)**		**Position**
	0.0		To MS
	13		To waste
MS Ionization Mode: API-ES, Positive and negative ion mode			
MS Target Ions	**Compound**		**Mass (m/z) and mode**
	BPA		227, – ion
	BHT		219, – ion
	FP		114, + ion
	BPU		197, + ion

Table IB. Approximate MS Conditions

General MS Parameters	Value
Ionization Mode	API-ES, Positive and negative Q1, multiple ions
Curtain Gas	30.00
Source Temperature	400 (pos), 450 (neg)
GS1	60 (pos), 50 (neg)
GS2	50
Resolution	Unit
Ion Source Voltage	+4500.00 (pos), –4500.00 (neg)

Compound Specific Parameters

Compound	Q1 Mass (*m/z*)	Polarity	Dwell time (s)	DP*	EP*
BPA	277	Neg	200	–90	–10
BHT	219	Neg	200	–90	–10
FP	144	Pos	200	105	10
BPU	197	Pos	200	105	10

*DP = Declustering potential; EP = Entrance Potential in volts (V).

calibration standard, prepared at 200 ppb, was used in the second analytical event.

Simulated material extracts were generated in a similar manner via a similar dilution process. However, in the case of the simulated material extracts, the test samples were prepared with the final dilution being performed with by one of three solutions, a pH 2 preparation (0.01 M HCl, 0.01 M KCl), 0.9% sodium chloride (saline), and a pH 8 buffer (0.065 M sodium monohydrogen phosphate and 0.0045 M potassium dihydrogen phosphate). These three types were chosen as they represent viable extraction media which can be used to simulate the interaction between an aqueous pharmaceutical solution and a container closure system (or material therein).

Performance evaluation experiments

The evaluation experiments were performed over the course of four separate analytical runs, performed as two separate analytical events (E1 and E2) on different days, with different analysts and with different chromatographic columns. The separate analytical events were similar but not equivalent. For example, as noted previously, a fifth calibration standard and spike level was used in event E2. Additionally, inter- and intra-run precisions were assessed at the 300 ppb level in event E1 and at 100 ppb in event E2. Finally, E2 did not include an assessment of performance in a saline matrix. Differences in the exact design of E1 and E2 did not result from the need or desire to investigate specific performance issues but reflect minor changes in tactics between events separated by a certain period of time.

The various performance characteristics were assessed in the following manner. Response versus concentration profiles were established in the standard and sample matrices by injecting each of the standards and samples in triplicate in each run. The resultant peak area response versus analyte concentration data was correlated using a linear regression model. Curve fit parameters, including the correlation coefficient (*r*) were obtained for these regression analyses. These linearity experiments were performed over the approximate range of 50 to 300 ng/mL and included a standard blank containing none of the target analytes.

Analytical accuracy was assessed as the ability to recover the analytes in the simulated extract test samples. The concentration of the analytes in the test samples was determined using calibration curves generated with the working calibration standards. Accuracy was calculated by comparing the determined concentration (C) of the analytes with their preparation target (T):

$$\text{Accuracy (\% recovery)} = (C/T) \times 100\%$$

Accuracy was assessed at three (or four) concentration levels spanning the calibration range.

Both intra- and inter-run precision was assessed by making replicate injections of the test samples in multiple analytical runs and was calculated as the percent relative standard deviation of the resultant peak area ratios. Intra-run precision (reproducibility) was established via six replicate injections of a test sample containing approximately 300 ng/mL (E1) or 100 ppb (E2) of the analytes. Test samples at other concentration levels were injected in triplicate within each run. Intra-run (intermediate) precision was established by performing this experiment in two separate analytical runs. In both cases, precision was calculated as the percent relative standard deviation (%RSD) of the determined analyte concentrations for all replicates.

Sensitivity was addressed via the calculation of the limit of quantitation (LOQ). The LOQ calculation was based on a signal-to-noise (S/N) evaluation of the response of the lowest concentration calibration standard (S/N ratio of 10).

Sample/standard stability was addressed in the following manner. Standards and test samples containing 300 ng/mL of the analytes were injected in replicate at the beginning of an extended analytical run. Additional portions of these samples were filled into autosampler vials that were placed at the end of analytical sequence. As the end of the analytical sequence approached, fresh preparations of these standards and samples were made and these preparations were injected into the chromatographic system at the end of the sequence along with the previously-filled vials of the original sample and standard preparations. Utilization of the

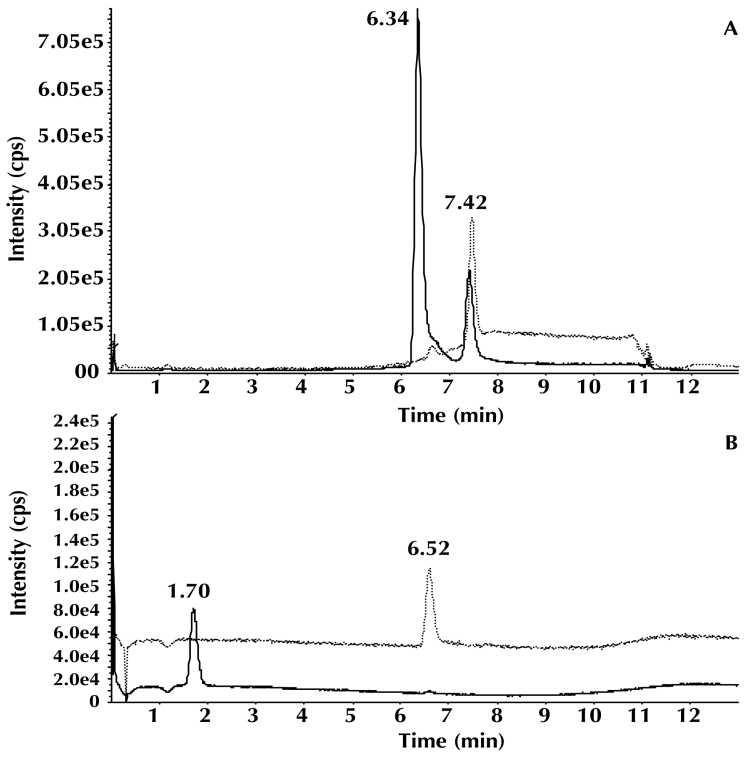

Figure 2. LC–MS extracted ion chromatograms for standard solutions. Figure 2A is the negative ion mode, where the chromatogram with the retention time of 6.34 min is BPA (*m/z* = 227) and the chromatogram with the retention time of 7.42 min is BHT (*m/z* = 219). The standard contained 300 ng/mL of these analytes. Figure 2B is for the positive ion mode, where the chromatogram with the retention time of 6.52 min is for BPU (*m/z* = 197) and the chromatogram with a retention time of 1.70 min is for FP (*m/z* = 114). The standard contained 50 ng/mL of these analytes.

freshly prepared standards allows one to differentiate between system response differences, beginning of run versus end of run, and sample/standard instability.

The sample and standard stability was calculated from the beginning of run response (BOR) and end of run (EOR) response for the standards and samples as follows. Firstly, the EOR for the stored sample or standard is corrected for any change in system response via the BOR of the calibration standard and the EOR of the freshly prepared standard:

$$\text{CS EOR} = \text{MS EOR} \times \left(\frac{\text{BOR, standard}}{\text{EOR, fresh standard}} \right)$$

where CS = Corrected sample and MS = Measured sample.

The stability ratio was calculated as the fractional change in the sample response:

$$\text{Stability ratio} = \frac{(\text{BOR, sample} - \text{corrected EOR, sample})}{\text{BOR sample}}$$

System suitability

The following tests were performed in each of the analytical runs as a means of assessing system suitability. Precision was assessed by calculating the %RSD of six replicate injections of a standard at a target concentration of 100 ng/mL of the analytes. Sensitivity was assessed as the signal to noise ratio obtained for the lowest concentration calibration standard (target concentration = 50 ng/mL). Linearity was assessed via duplicate injections of the calibration standards. Response stability was assessed by making injections of the highest concentration standard throughout the course of an analytical run and calculating the %RSD of the obtained analytical responses.

Each aspect of the performance evaluation was performed twice as independent events. These two independent evaluations are termed E1 and E2 throughout this manuscript.

Results and Discussion

Method development

The chromatographic separation was optimized so that the analytes of interest were retained on the column sufficiently long to be free from any void volume effects but not so long as to produce an excessive run time, and the analytes were resolved from one another and other anticipated sample components. This latter requirement is not unilateral as MS detection provides sufficient specificity that complete chromatographic separation is not necessary.

Typical selected ion chromatograms for standard solutions are shown in Figure 2. The individual analytes produce well-resolved chromatographic peaks whose magnitude is well above the chromatographic noise (signal to noise ratio greater than 10). The negative ion chromatogram at *m/z* of 227 contains a major peak

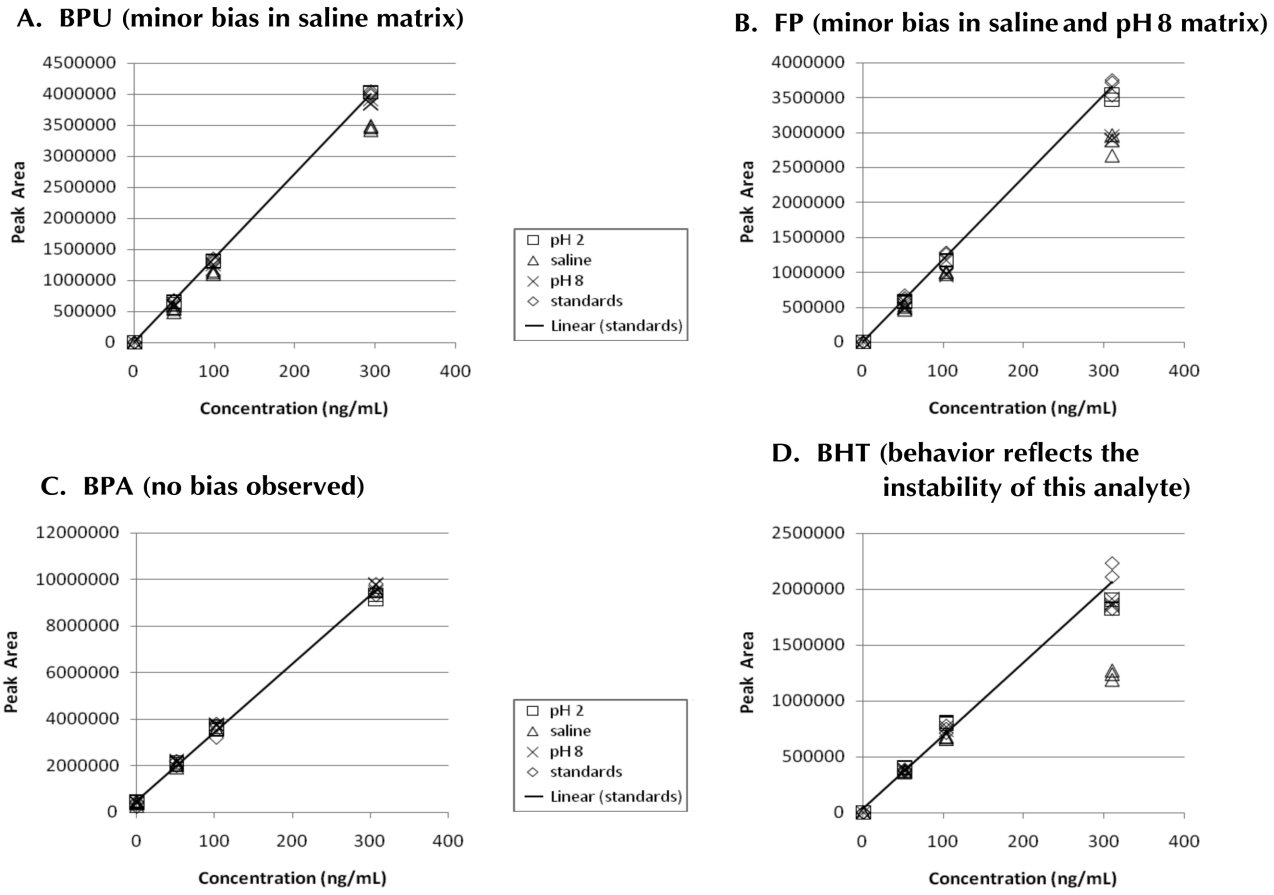

Figure 3. Calibration curves for calibration standards and spiked test samples, prepared in the various simulated extracting media. The comparison of the individual data points for the samples versus the best-fit line for the standards reflects the magnitude of sample matrix-related bias. The calibration curves were not forced through the origin.

for the target analyte (BPA) and a minor system peak that elutes at approximately the same time as BHT. This minor peak was present in every injection, regardless of sample type. The genesis of this peak was not established since the peak had no practical analytical impact as it is well resolved from BPA at *m/z* of 227 and it was not present at *m/z* of 219 (and therefore did not interfere with BHT).

Method evaluation

Response function (linearity)

Plots of analyte response versus prepared concentrations in test samples and standards are shown in Figure 3. The response functions for BPA, BPU, and FP are well-behaved and, as shown in Table II, correlation coefficients obtained in all the analytical matrices were generally greater than 0.9990. Additionally, the response plots show neither large or systematic differences in the responses obtained in the various sample matrices, indicating that method response is not materially impacted by sample composition for the matrices studied. This suggests that the method will be appropriately accurate for these three analytes in all three extraction solvents.

Accuracy

Accuracy results, presented as the % spike recovery, are summarized in Table III. In general the recoveries were in the range of 70–110% for all analytes and all extracting matrices. Such performance is within generally accepted requirements for extractables quantitation at low (sub ppm) levels (19–21).

Precision

Intra-run precision (reproducibility) was assessed via six sequential injections of working samples containing approximately either 300 ng/mL (E1) or 100 ppb (E2) of the target analytes and via three sequential injections of working samples at the other concentration levels. The inter-run (intermediate) precision was assessed by repeating the intra-run precision experiment in a second analytical run.

The precision results are shown in Table IV. Intra-run precision was excellent, typically 5% RSD or less and the inter-run precision was less than 10%. Both of these outcomes met the desired performance levels.

Table II. Results of the Linearity Assessment, Correlation Coefficients (r)

Analyte	Evaluation Run	Correlation Coefficient, r (linear response function)*			
		Standards	Saline Sample	pH 2	pH 8
BPA	E1	0.9991	0.9997	0.9995	0.9993
	E2	0.9981	–†	0.9978	0.9981
FP	E1	0.9999	0.9993	0.9999	0.9999
	E2	0.9998	–†	0.9997	0.9998
BPU	E1	0.9999	0.9994	0.9999	0.9997
	E2	0.9996	–†	0.9994	0.9996

* Corresponding to solutions prepared to contain 0, 50, 100 and 300 ng/mL of the analytes in E1. A fifth standard, 200 ng/mL, was used in E2.
† The performance of the assay in a saline matrix was not assessed in a second experiment.

LOQ

Calculated LOQ values (based on 10 times the signal to noise for the lowest concentration standards) were 8, 3, and 5 ng/mL respectively for BPA, FP, and BPU. Somewhat improved sensitivity was achieved in the second analytical event and the calculated LOQs were closer to 1 ng/mL for each analyte. As was noted previously, adequate accuracy and precision data were obtained for the lowest concentration test sample containing approximately 50 ng/mL of each analyte. It was therefore concluded that the method is capable of producing accurate and precise quantitation for the target analytes at concentrations as low as 50 ng/mL and can be used to estimate concentration at levels as low as 10 ng/mL.

Sample and standard stability

Stability was addressed over the course of an analytical run lasting 25 h. The stability ratios obtained (reflecting only the change in response due to sample instability and not any changes due to detector drift) were 0.20 or less for BPA, FP, and BPU in all the sample matrices, reflecting adequate performance. Given the previously noted instability of BHT, it is not unexpected that the stability ratios for this analyte were greater than 0.5.

BHT

The results for this analyte were characterized by MS responses that decreased noticeably over the course of the analytical runs.

Table III. Accuracy Results of BPA, FP, and BPU

Target Conc. (ng/mL)	Evaluation Run	Accuracy (Mean % Recovery)*					
		pH 2 Matrix		Saline Matrix		pH 8 Matrix	
		Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
Accuracy Results, BPA							
50	E1	106%	A†	100%	A†	112%	A†
	E2	111%	A†	B‡	B‡	118%	A†
100	E1	104%	A†	104%	A†	110%	A†
	E2	101%	105%	B‡	B‡	115%	114%
200	E2§	99.9%	A†	B‡	B‡	110%	A†
300	E1	100%	94.4%	104%	98.1%	105%	96.8%
	E2	96.9%	A†	B‡	B‡	107%	A†
Accuracy Results, FP							
50	E1	91.1%	A†	79.5%	A†	77.9%	A†
	E2	97.0%	A†	B‡	B‡	103%	A†
100	E1	94.8%	A†	80.2%	A†	77.8%	A†
	E2	99.1%	101%	B‡	B‡	101%	96.5%
200	E2§	97.1%	A†	B‡	B‡	100%	A†
300	E1	93.9%	101%	75.2%	90.0%	78.1%	89.7%
	E2	99.7%	A†	B‡	B‡	102%	A†
Accuracy Results, BPU							
50	E1	104%	A†	86.3%	A†	99.2%	A†
	E2	102%	A†	B‡	B‡	105%	A†
100	E1	106%	A†	91.8%	A†	101%	A†
	E2	99.5%	97.9%	B‡	B‡	104%	99.1%
200	E2§	96.4%	A†	B‡	B‡	102%	A†
300	E1	109%	102%	94.9%	94.2%	105%	95.9%
	E2	101%	A†	B‡	B‡	105%	A†

* Desired performance: 100 ± 30%.
† A = Samples at this concentration were not tested in this run.
‡ The saline matrix was not tested in the E2 evaluation event.
§ Samples prepared at a level of 200 ppb were only tested in the E2 evaluation event.

Because such a large decrease in MS response was not exhibited by the other analytes and because the UV response of BHT also decreased with time, it was concluded that this phenomenon was not an analytical issue (for example, MS detector instability) but rather an analyte stability issue (that is, this analyte was degrading over time in certain of the sample matrices). Thus although the apparent performance of the method was adequate over the short term (for example, all injections related to a certain sample type), apparent method performance was poor over the long term. This is illustrated, for example, in Figure 3D, the calibration plots for BHT in the four analytical matrices (water matrix for standards, and the three extracting solutions). A clear decline in analytical response was observed across the various sample matrices. Although this could be construed to be a sample matrix effect, it is the case that the magnitude of the decline reflects the order in which the sample matrices were injected (and thus how long they were stored prior to injection). Thus the response to the samples that were injected at the end of the run (high concentration saline matrix) was clearly reduced versus the samples injected at the beginning of the run (standards).

Another indication of the sample stability issue was precision. Reproducibility, a short-term performance characteristic defined as the imprecision of sequential injections and captured as the % relative standard deviation (%RSD), was 5% or less for all analyte concentrations and all sample matrices. Alternatively, response stability, a long-term performance characteristic defined as the imprecision of all injections of a particular sample (in this case a standard at 300 ppb) made over the course of an analytical run, was 16% for both analytical runs.

Because an analytical run is typically constructed of multiple groups of samples bracketed by standards, the effect of changing response on accuracy can be mitigated somewhat if the accuracy data is processed on a bracket by bracket basis and not on the basis of the entire run being a single bracket. Thus in most cases, the analytical accuracy (% spike recovery) for BHT fell within the range of 97–128% for all analyte levels and for all sample matrices. This suggests that the method is intrinsically accurate for BHT quantitation. However, an apparent analytical accuracy of 66% was obtained for the high concentration spike of the saline matrix, as it is at the high concentration in the saline matrix that the analyte is least stable. An LOQ of 12 ng/mL was calculated for BHT.

Table IV. Precision Results BPA, FP, BPU

Target Conc. (ng/mL)	Eval Run	Precision (% RSD)*					
		pH 2 Matrix		Saline Matrix		pH 8 Matrix	
		Reprod††	Intermed††	Reprod††	Intermed††	Reprod††	Intermed††
Precision Results, BPA							
50	E1	3.2%	A†	5.0%	A†	2.1%	A†
	E2	2.6%	A†	B‡	B‡	3.7%	A†
100§	E1	2.2%	A†	0.2%	A†	0.8%	A†
	E2	3.6% (2.4%)	3.5%	B‡	B‡	2.5% (1.4%)	2.0%
200**	E2	0.9%	A†	B‡	B‡	1.6%	A†
300§	E1	1.2% (1.5%)	3.1%	0.4% (1.0%)	2.9%	1.9% (1.1%)	4.5%
	E2	2.6%	A†	B‡	B‡	1.1%	A†
Precision Results, FP							
50	E1	1.3%	A†	6.9%	A†	2.2%	A†
	E2	2.5%	A†	B‡	B‡	1.2%	A†
100§	E1	0.5%	A†	1.7%	A†	1.5%	A†
	E2	1.7% (1.3%)	2.1%	B‡	B‡	1.0% (2.0%)	1.5%
200**	E2	2.3%	A†	B‡	B‡	1.4%	A†
300§	E1	1.2% (3.2%)	3.2%	4.1% (1.6%)	9.8%	1.2% (0.8%)	7.2%
	E2	0.7%	A†	B‡	B‡	1.4%	A†
Precision Results, BPU							
50	E1	3.4%	A†	6.7%	A†	3.2%	A†
	E2	0.8%	A†	B‡	B‡	2.3%	A†
100§	E1	0.7%	A†	2.5%	A†	3.3%	A†
	E2	0.8% (0.7%)	1.1%	B‡	B‡	1.5% (1.2%)	2.6%
200**	E2	2.4%	A†	B‡	B‡	1.7%	A†
300§	E1	0.5% (0.5%)	3.4%	2.0% (1.0%)	1.5%	1.0% (1.4%)	4.9%
	E2	0.7%	A†	B‡	B‡	2.0%	A†

* Desired performance: Reproducibility, not more than (NMT) 10%; Intermediate, NMT 15%. Reproducibility based on $n = 3$ or $n = 6$ (intermediate precision concentration only); Intermediate based on $n = 12$ at either 100 ng/mL or 300 ng/mL (6 each in E1 and E2).
† A = Samples at this concentration were not tested in the second analyst run.
‡ B = The saline matrix was not tested in the E2 evaluation.
§ The first value is for analyst 1 and the second value in ( ) is for analyst 2.
** Samples prepared at a level of 200 ppb were only tested in the E2 evaluation.
†† Reprod = Reproducibility and Intermed = Intermediate.

System suitability

Method qualification confirms that a properly implemented method will produce information of known and acceptable quality. Method qualification does not, however, provide any assurance that a method is appropriately implemented at its time of use. Rather, such assurance is obtained via the system suitability assessment. System suitability testing consists of two aspects, performing a specified series of actions to collect performance data and comparing that performance data to requirements that presumably differentiate between a system that is capable of producing valid data and one that is not.

System suitability tests for inter-run precision, magnitude of response (signal to noise), response stability and calibration curve linearity were performed in each of the two runs performed in this study. These system suitability results, illustrated in Table V, were used to establish acceptance criterion (also in Table V) that would need to be met in order for subsequent runs of this method to be deemed to be acceptable. It is noted that the acceptance criterion for response stability is set so that BHT does not pass the criterion, consistent with its known stability issues.

Conclusion

An LC–MS has been developed for the purpose of quantitating the four target extractables at low concentrations in aqueous extracting media with a pH between 2 and 8. The developed method's performance was established by assessing performance

Table V. System Suitability Data and Adopted Acceptance Criteria

	BPA				BHT			
Run #	Precision*	Signal to Noise†	Linearity‡ (*r*)	Response Stability§	Precision*	Signal to Noise†	Linearity‡ (*r*)	Response Stability§
1	1.3%	115	0.9991	6.1%	2.0%	25	0.9990	16%**
2	3.2%	108	0.9989	2.9%	5.3%	8 (5)	0.9940	16%**
3	1.1%	299	0.9970	6.9%	N/A††	N/A††	N/A††	N/A††
4	2.5%	1490	0.9972	3.0%	N/A††	N/A††	N/A††	N/A††
	FP				**BPU**			
Run #	Precision*	Signal to Noise†	Linearity‡ (*r*)	Response Stability§	Precision*	Signal to Noise†	Linearity‡ (*r*)	Response Stability§
1	1.4%	100	0.9999	2.6%	0.8%	44	0.9999	15%
2	2.1%	132	0.9995	2.1%	0.5%	126	0.9996	2.4%
3	0.9%	365	0.9997	5.0%	2.4%	562	0.9996	3.7%
4	1.5%	574	0.9998	1.3%	1.3%	593	0.999	2.3%
Criteria								
	NMT 10%	≥ 10	NLT‡‡ 0.9900	NMT 15%	NMT 10%	≥ 10	NLT 0.9900	NMT 15%

* %RSD of 6 sequential injections of an intermediate concentration standard made at the beginning of a run.
† Measured using the lowest concentration standard containing » 50 ng/mL.
‡ Obtained from duplicate injections of the calibration standards.
§ % RSD of all injections made of an intermediate concentration standard throughout the course of a run.
** These results represent system suitability failures, due primarily to the instability of BHT.
†† Efforts to quantitate BHT did not continue into runs 3 and 4.
‡‡ NLT = not less than.

characteristics including accuracy, response linearity, precision, specificity, and solution stability. The developed method is suitable for the quantitation of three of the analytes, BPA, FP, and BPU, in the concentration range of 50 to 300 ng/mL and may be used to produce concentration estimates for these analytes to a concentration as low as 10 ng/mL. Although the developed method appears to be suitable for the quantitation of BHT at similar concentrations, the instability of this analyte in samples and standards means that the analytical runs to quantify this analyte will have to include frequent standard brackets.

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