Development and Validation of an HPLC–MS–MS Method For Quantitating Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (Tinuvin 770) and a Related Substance in Aqueous Extracts of Plastic Materials

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Abstract

Tinuvin 770 is a light stabilizer present in numerous polymers utilized in medical or pharmaceutical applications (e.g., manufacturing, packaging, delivery systems and devices). Under conditions of use, Tinuvin 770 and its related substances may leach from the polymers and accumulate in pharmaceutical products that are administered to subjects to produce a therapeutic benefit. In order to establish the amounts of Tinuvin 770 that may be extracted from such systems and devices, sensitive and selective analytical methodologies are required. A liquid chromatographic method with tandem mass spectrometric detection (LC–MS–MS; API-ES, positive ion mode) has been developed for the purpose of quantitating Tinuvin 770 and a related substance at low concentrations [200 ng/mL (ppb) or less] in aqueous extracting media. Issues related to injection-to-injection carryover and sample matrix effects were mitigated by the addition of potassium chloride to the test samples, where the potassium ion increases Tinuvin solubility via a "salting in" effect. The developed method was validated for this application by assessing performance characteristics including accuracy, response linearity, precision, specificity, and solution stability. The validated method is suitable for the quantitation of these analytes in the concentration range of 1–200 ng/mL.

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 and desirable performance characteristics. While an important performance characteristic of plastics used in medical/pharmaceutical applications is chemical inertness, interactions between a plastic material and the pharmaceutical product it comes in contact with are well-documented. 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, photostabilizers are plastic additives that retard or prevent their light-induced degradation. Hindered amine light stabilizers (HALS) are an important group of such additives, and bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate (commercially known as HALS 770, HS 770, JF 90, LA 77, LA 77Y, LS 770, Lowilite 77, Mark LA 77, NF 90, Sanol, Sanol 770, Sanol LS 700, Sanol LS 770, Sumisorb 577, T 770, TIN 770, TN 770, Tinuvin 770, Tinuvin 770DF, Tinuvin 770DF1, Tinuvin 770LS, Uvinul 4077, and Viosorb 04) (Figure 1) is a HALS that is used worldwide in polyethylene, polypropylene, polycarbonate, polyurethane, polyamide, polyacetyl, acrylonitrile, and polyisoprene polymers. Use of such polymers in pharmaceutical systems and devices presents the possibility that Tinuvin 770 (and any related substances such as impurities and decomposition

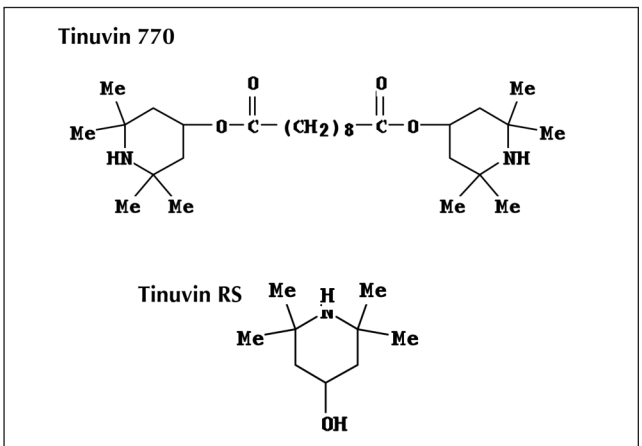

Figure 1. Chemical structures of the analytes of interest. Tinuvin 770: Bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate, CAS RN # 52829-07-9, $C_{28}H_{52}N_2O_4$, molecular weight = 480.72. Tinuvin RS: 2,2,6,6-Tetramethyl-4-piperidinol, CAS RN #2403-88-5, $C_9H_{19}NO$, molecular weight = 157.25.

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products) could leach from the system or device into a pharmaceutical product that is then administered to a patient in a clinical situation.

Accumulation of leached Tinuvin 770 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. 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. In the case of Tinuvin 770, safety is a pertinent concern as this compound has a documented toxicological risk (1–4).

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. In the case of Tinuvin 770 specifically, methods such as high-performance liquid chromatography (HPLC) with various detection strategies [e.g., liquid chromatography–UV detection (5–7), liquid chromatography–mass spectrometry (8), liquid chromatography–evaporative light scattering detection (5)], gas chromatography (5,9,10), coupled liquid and gas chromatography (11–13), and thin layer chromatography (14)] have been utilized to detect and/or quantify this analyte in polymer extracts. While these methods are generally applicable to a number of analytical situations involving characterization of materials (and/or their associated extracts) for Tinuvin 770, many of these methods included either relatively labor-intensive sample preparation processes or were not sufficiently sensitive for certain applications. Consider, for example, the accumulation of Tinuvin 770 in aqueous parenteral products stored in a container-closure system that includes a component that contains this additive. In such circumstances, the sample matrix is relatively simple thus precluding the need for complicated sample preparation steps, but the anticipated concentrations of the analyte are low, in the sub-ppm range. Additionally, it may be that the substances that are extracted from the container closure system include not only Tinuvin 770 but also its related substances, such as impurities and decomposition products.

Thus, an analytical method was developed to allow for the quantitation of Tinuvin 770 and its related substance (T-RS: 2,2,6,6-Tetramethyl-4-piperidinol) (Figure 1) in aqueous material extracts at concentrations of less than 100 ng/mL (parts-per-billion, ppb). The developed method, liquid chromatography with tandem mass spectrometry (LC–MS–MS) detection, was then validated, consistent with published recommendations for the validation of extractable/leachables assays (15–17).

Experimental

Chemicals and reagents

Solvents and chemicals were obtained commercially in the highest appropriate purity. Tinuvin 770 (T-770), bis(2,2,6,6-tetramethyl-4-piperidyl)sebacate, and diethyl phthalate (DEP, internal standard) were obtained from Sigma Aldrich (St. Louis, MO). The Tinuvin-related substance (T-RS, 2,2,6,6-tetramethyl-4-piperidinol) was obtained from Fluka (St. Louis, MO). HPLC-grade methanol was obtained from Honeywell Burdick and Jackson (Morristown, NJ). HPLC-grade ammonium acetate was obtained from Aldrich. Potassium chloride was obtained from Mallinckrodt (Phillipsburg, NJ). Laboratory-grade water was used throughout this study.

HPLC System

The chromatographic system was an Applied Biosystems 4000 Q Trap MS–MS detector (Foster City, CA) coupled to an Agilent 1200 HPLC system consisting of a binary pump (G1312A), refrigerated autosampler (G1329A. G1330B), thermostatted column compartment (G1316A), degasser (G1379B), and diode array detector (G1315B) (Santa Clara, CA). The data was acquired and analyzed via a Dell Precision 390 Workstation using Applied Biosystems Analyst 1.4.2 software (Round Rock, TX). The chromatographic column was a Waters XTerra MS C_{18} (30 × 4.6 mm, 2.5 µm particles, P/N 186000600) (Milford, MA).

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 of the analytes and internal standards (Table II). Stock solutions of these compounds were prepared at a nominal concentration of 1000 µg/mL by dissolving the reagent in methanol. A composite analyte stock solution, containing 5 µg/mL T-RS and 10 µg/mL Tinuvin 770, was prepared by dilution of the individual analyte stock solutions with methanol. An intermediate standard stock was prepared at appropriate concentrations by dilution of the composite stock with water and adding sufficient methanol to maintain a methanol level of 20%. Working calibration standards were prepared by adding portions of the inter-

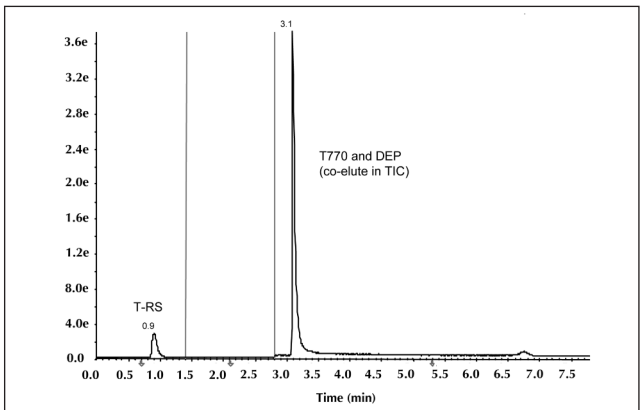

Figure 2. LC–MS total ion current (TIC) chromatogram for a standard containing 5 ng/mL T-RS, 10 ng/mL Tinuvin 770, and 400 ng/mL of the DEP internal standard. Note that while Tinuvin 770 and DEP co-elute chromatographically, they are "resolved" in detection due to the selected ion monitoring (SIM) MS–MS detection used.

Table I. LC–MS–MS Conditions

Parameter		Value	
Column		Waters Xterra MS C_{18}, 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	
Gradient	**Time (min)**	**Flow Rate (mL/min)**	**Proportion B (%)**
	0	0.8	5
	0.2	0.8	5
	1.5	0.8	98
	5.0	0.8	98
	5.1	0.8	5
	8.0	0.8	5
Needle wash	*Wash needle three times in methanol (three separate vials)*		
Diverter Valve		**Total Time (min)**	**Position**
		0.6	To MS
		7.5	To waste
MS Ionization Mode	*API-ES, Positive Q1 and Q3 MRM*		
MS Target Ions	**Compound**		**Q1/Q3 Mass (*m/z*)**
	Tinuvin 770		481.3/140.1
	T-RS		158.1/58.0
	DEP (internal standard)		223.1/149.1

Table II. Preparation of Calibration Standards

Standard	mL of Intermediate Stocks A–D*	mL of DEP IS†	mL of 0.10 M KCl	Dilution Volume (mL)‡	Approx. Conc. (ppb)
S1	1.0 of A	1.0	1.0	10.0	5 (T-RS) or 10 (T-770)
S2	1.0 of B	1.0	1.0	10.0	10 (T-RS) or 20 (T-770)
S3	1.0 of C	1.0	1.0	10.0	30 (T-RS) or 60 (T-770)
S4	1.0 of D	1.0	1.0	10.0	100 (T-RS) or 200(T-770)
S0	1.0 of E§	1.0	1.0	10.0	N/A

* Stock solutions containing ~ 1000 µg/mL of each compound were prepared. A composite stock containing ~ 5 or 10 µg/mL of each analyte was prepared by diluting portions of the individual stocks with methanol. Intermediate standard stocks A, B, C, and D were prepared by diluting 1.0, 2.0, 6.0, or 20.0 mL of the composite stock to 100 mL with water, adding enough methanol to bring the organic content to 20%.
† The diethyl phthalate internal standard stock was prepared to contain ~ 4 µg/mL DEP in methanol.
‡ Diluent = water. § E = 10% Methanol.

Table III. Preparation of Test Samples

Test Sample ID	mL of DEP Internal Standard	mL of Intermediate Stocks A–D*	Dilution Volume (mL)†	Conc. of each analyte (ppb)
C1	1.0	1.0 of A	10.0	5 (T-RS) or 10 (T-770)
C2	1.0	1.0 of B	10.0	10 (T-RS) or 20 (T-770)
C3	1.0	1.0 of C	10.0	30 (T-RS) or 60 (T-770)
C4	1.0	1.0 of D	10.0	100 (T-RS) or 200(T-770)
C0	1.0	1.0 of E‡	10.0	N/A

* See Table II for a description of these intermediate stocks.
† Used either neutral water, pH 2, or pH 8. For neutral water solutions, added 1.0 mL of 0.1 M KCl.
‡ E = 10% methanol.

mediate stocks and the internal standard stock and diluting the mixture with water.

Simulated material extracts were generated in a similar manner via a similar dilution process (Table III). However, in the case of the simulated material extracts, the test samples were prepared with the final dilution being performed with one of three solutions, a pH 2 preparation (0.01 M HCl, 0.01 M KCl), water, 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 that can be used to simulate the interaction between a pharmaceutical solution and a container closure system (or material therein).

Validation experiments

The validation experiments were performed over the course of four separate analytical runs on different days with two different analysts and with two different lots of chromatographic columns. The various performance characteristics were assessed in the following manner. Response versus concentration profiles were established in the standard and sample matrices profiles in two separate analytical runs by injecting each of the standards and samples identified in Tables II and III in triplicate in each run. The resultant peak response versus analyte concentration data was correlated using a quadratic 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 10–200 ng/mL for T-770 and 5–100 ng/mL for T-RS.

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):

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

As noted in Table III, accuracy was assessed at four concentration levels spanning the calibration range.

Both inter-run and intra-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 (%RSD) of the resultant peak area ratios. Inter-run precision was established via six replicate injections of a test sample containing approximately 60 ng/mL T-770 and 30 ng/mL T-RS. Test samples at other concentration levels were injected in triplicate within each run. Intra-run precision was established by performing this experiment in four separate analytical runs. In both cases, precision was calculated as the %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 30 ng/mL T-770 and 60 ng/mL T-RS 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 the 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 freshly prepared standards allows one to differentiate between system response differences, beginning-of-run (BOR) versus end-of-run (EOR), and sample/standard instability.

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

Corrected sample EOR = Measured sample EOR × (BOR, standard/EOR, fresh standard)

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

Stability ratio = (BOR, sample – corrected EOR, sample)/ BOR sample

Table IV. Validation Acceptance Criteria

Parameter	Acceptance Criterion
Linearity	$r \geq 0.99$
Accuracy	Recovery of 100 ± 30% at concentrations greater than 10 ng/mL, 100 ± 40% at concentration of 10 ng/mL or less. Applicable for each individual injection and for the mean of all injections.
Inter-run precision	%RSD of six replicate injections of S3 ≤ 10%.
Intra-run precision	%RSD of six replicate injections of S3 made in each run for multiple runs ≤ 15%.
LOQ	LOQ ≤ 5 ng/mL for T-RS, 10 ng/mL for T-770.
Sample Stability	Absolute value of the stability ratio ≤ 0.2

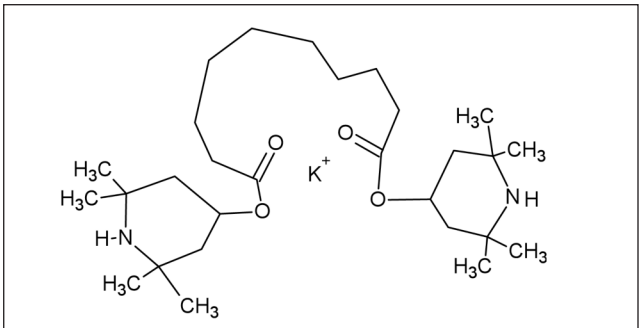

Figure 4. Proposed structure of the potassium-tinuvin complex responsible for the increased solubility of Tinuvin and the associated analytical benefits.

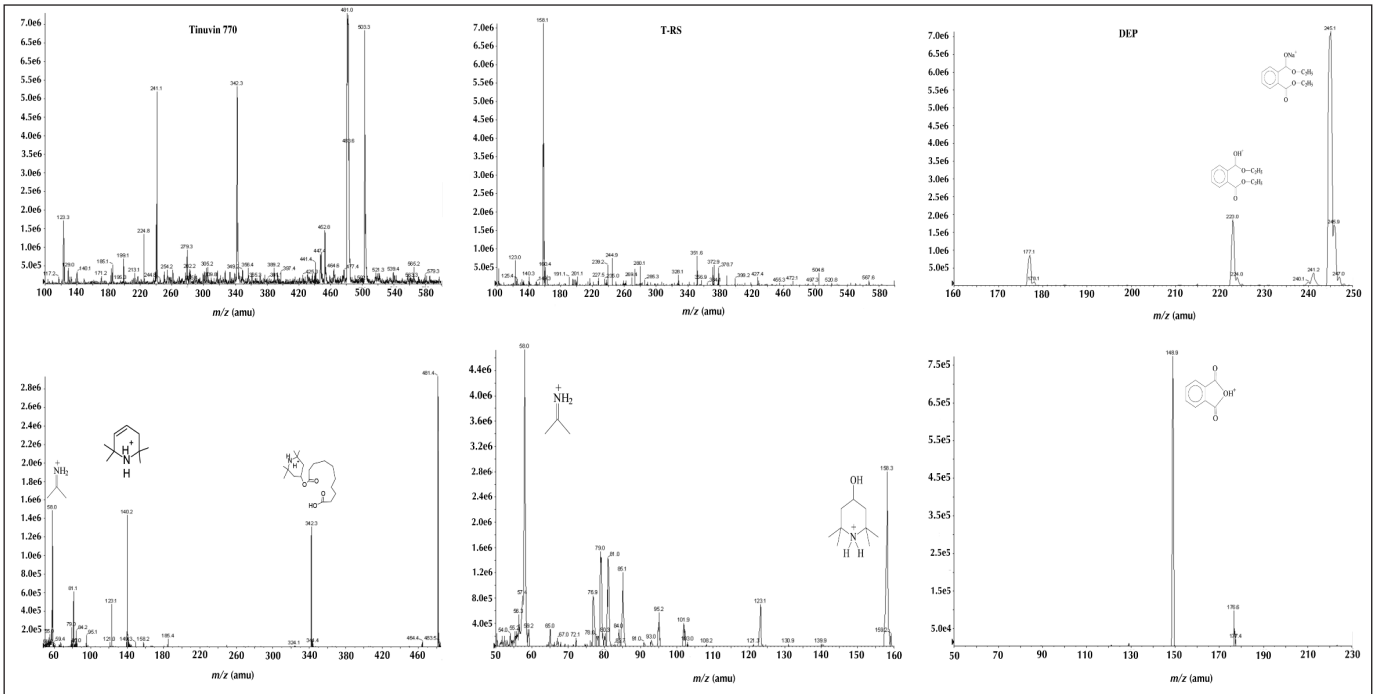

Figure 3. Q1 MS spectra (upper plot) and Q3 MS–MS spectra (lower plot) for the internal standard and the analytes of interest, justifying the choice of the MS–MS detection conditions adopted in the validated method.

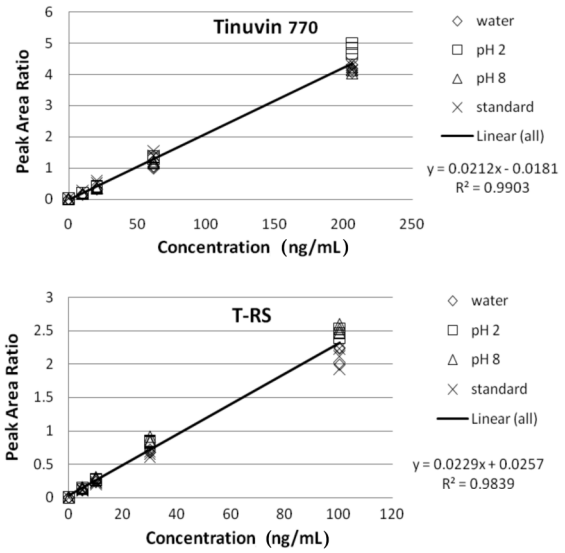

Figure 5. Linear response curves for both calibration standards, prepared in water, and spiked test samples, prepared in the various simulated extracting media. The illustrated best-fit line reflects the total population of all sample and standard solutions data, which demonstrates that the developed method exhibits little or no matrix-related bias. The slight albeit visually indistinguishable curvature in the response curves is more effectively modeled by using a quadratic function, which is therefore recommended for analyte quantitation and was used in the accuracy assessment performed in this study.

Table V. Results of the Linearity Assessment and Correlation Coefficients (r)

	Correlation Coefficient, *r* (quadratic response function)*							
	Standards		Water sample		pH 2 Sample		pH 8 Sample	
Analyst	T-770	T-RS	T-770	T-RS	T-770	T-RS	T-770	T-RS
1	0.9995	0.9983	0.9995	0.9977	0.9994	0.9996	0.9996	0.9996
2	0.9999	1.0000	–†	–†	0.9998	0.9999	0.9996	0.9991

* Acceptance criterion: r ≥ 0.99. † Water matrix samples were not tested in run 2.

Table VI. Accuracy Results for pH 2 Solution Matrix

	Tinuvin 770					T-RS			
Target ng/mL	% Recovery*				Target ng/mL	% Recovery*			
	Run 1	Run 2	Run 3	Run 4		Run 1	Run 2	Run 3	Run 4
10	73.8	†	86.0	†	5	99.8	†	104	†
	73.5	†	84.0	†		99.9	†	119	†
	76.8	†	88.4	†		99.5	†	120	†
20	97.7	†	83.7	†	10	101	†	110	†
	99.8	†	89.4	†		97.0	†	113	†
	103	†	91.2	†		100	†	112	†
60	115	106	96.2	130	30	101	98.0	120	99.4
	112	110	100	124		97.9	98.3	116	106
	107	109	104	120		101	99.1	118	97.9
	109	110	102	119		100	98.4	120	107
	106	109	102	122		101	99.1	122	112
	101	108	104	121		98.8	99.0	114	108
200	105	†	117	†	100	97.9	†	105	†
	104	†	122	†		95.1	†	112	†
	107	†	127	†		94.9	†	109	†

* Acceptance criterion: 100 ± 30% for all but lowest analyte concentration, which is 100 ± 40%.
† Replicates at this concentration were not tested in this run.

Acceptance criteria

A cornerstone of the validation concept is that a validation is a quantitative exercise comparing measured performance to relevant, pre-specified acceptance criteria. The term "relevant" is particularly meaningful in the case of extractables and leachables quantitation as the targeted analytes are typically present at low concentrations in analytically challenging matrices. Thus, the acceptance criteria for methods used for extractables/leachables assessments are typically much less rigorous than the acceptance criteria for methods used to quantitate the active ingredient in pharmaceutical products.

The acceptance criteria used in this study, reflecting reasonable requirements and obtainable performance levels for this particular analytical situation, are summarized in Table IV.

System suitability

The following tests were performed in each of the four analytical runs as a means of assessing system suitability. Precision was assessed by calculating the %RSD of six replicate injections of a standard containing approximately 60 ng/mL T-770 and 30 ng/mL T-RS. Sensitivity was assessed as the S/N ratio obtained for the lowest concentration calibration standard (approximately 5 ng/mL T-RS and 10 ng/mL T-770). Linearity was assessed via duplicate injections of the calibration standards outlined in Table II. Response stability was assessed by making injections of a standard (the same one used for precision) throughout the course of an analytical run and calculating the %RSD of the obtained analytical responses.

Extraction

A synthetic polyisoprene material formulated with Tinuvin 770 was extracted and the resultant extracts were tested by the validated method. Specifically, 0.62 grams of material was extracted with 50 mL of pH 2, water, and pH 8 extracting media by autoclaving at 121°C for 30 min.

Results and Discussion

Method development

The chromatographic separation was optimized so that (A) the analytes of interest were retained on the column sufficiently long enough to be free from any void volume effects but not so long as to produce an excessively long run time and (B) the analytes were resolved from one another and other anticipated sample components. This latter requirement was not all that important in this application as MS–MS detection provides sufficient specificity that a complete chromatographic separation is not necessary. In fact, Tinuvin 770 and the internal standard co-elute under the operating conditions (Figure 2).

This is actually a desirable circumstance because the co-elution of one of the analytes and the internal standard implies that, in theory, the internal standard and the analyte experience the same separation/detection environment, thereby facilitating the internal standard's ability to mimic the analyte's behavior.

The MS and MS–MS mass spectra for the analytes and the internal standard are shown in Figure 3 and illustrate and justify the selection of the method's detection conditions.

Significant sample-to-sample carry over for Tinuvin 770 was observed in the initial stages of method development. Additionally, there was a marked difference in response as a function of sample matrix: samples prepared in the pH 2 and pH 8 extracting media producing larger responses than similar concentration samples prepared in either water or methanol/water mixtures. When it was demonstrated that this latter effect was not the result of a detection-based matrix enhancement effect, it was hypothesized that the presence of potassium ion in the sample matrix increased the solubility of the Tinuvin 770, a process termed "salting in" (18,19). Specifically, it is proposed that potassium ion forms a complex with Tinuvin 770, resulting in its increased solubility (Figure 4). In such a circumstance, the addition of potassium to all samples and standards would have the effect of reducing carry-over, subsequently producing an apparent increase in response as less analyte "sticks" to the system and more is eluted to the detector.

The solubility hypothesis was confirmed via quantitative experiments that demonstrated Tinuvin 770's increased solubility in the presence of potassium ion. More significantly from a chromatographic perspective, addition of KCl to the samples and standards mitigated but did not eliminate sample carry over and essentially eliminated any response differences related to sample matrix.

In order to reduce sample carry over further, injector needle washes with methanol between injections were increased from one to three (with each wash occurring from separate methanol-containing vials), and a "wash" injection (with methanol) was placed in-between sample injections.

Table VII. Accuracy Results for pH 8 Solution Matrix

Tinuvin 770					T-RS				
Target ng/mL	% Recovery* Run 1	Run 2	Run 3	Run 4	Target ng/mL	% Recovery* Run 1	Run 2	Run 3	Run 4
10	70.7	†	77.8	†	5	96.7	†	127	†
	70.3	†	78.6	†		94.5	†	127	†
	70.5	†	78.6	†		95.9	†	108	†
20	94.2	†	75.4	†	10	99.3	†	128	†
	88.9	†	79.8	†		98.6	†	124	†
	92.1	†	80.2	†		97.9	†	121	†
60	111	95.1	87.5	102	30	98.3	101	118	121
	110	96.0	88.6	104		99.5	98.9	129	130
	103	98.7	91.5	106		98.7	98.2	121	130
	106	97.6	92.1	107		100	101	129	115
	102	99.9	95.6	108		98.8	101	118	130
	96.3	98.0	97.9	109		98.4	98.4	125	128
200	101	†	99.4	†	100	99.2	†	112	†
	101	†	102	†		99.3	†	110	†
	104	†	105	†		102	†	115	†

* Acceptance criterion: 100 ± 30% for all but lowest analyte concentration, which is 100 ± 40%.
† Replicates at this concentration were not tested in this run.

Table VIII. Accuracy Results for Water Matrix

Tinuvin 770			T-RS		
Target ng/mL	% Recovery* Run 1	Run 2	Target ng/mL	% Recovery* Run 1	Run 2
10	68.2	†	5	101	†
	67.2	†		89.7	†
	66.4	†		95.7	†
20	72.1	†	10	107	†
	71.1	†		97.8	†
	70.3	†		97.3	†
60	74.1	82.9	30	95.1	111
	77.4	86.9		95.2	105
	79.1	90.0		101	105
	78.6	90.8		99.0	101
	78.2	93.6		101	110
	79.8	92.7		99.7	109
200	99.7	†	100	86.8	†
	103	†		98.0	†
	107	†		98.6	†

* Acceptance criterion: 100 ± 30% for all but lowest analyte conc.: 100 ± 40%.
† Replicates at this concentration were not tested in this run.

Method validation

Response function (linearity)

Plots of analyte response ratio versus prepared concentrations in test samples and standards are shown in Figure 5. While the response functions are well-behaved, the correlation between concentration and response is improved somewhat with the use of a quadratic model versus a linear model. As shown in Table V, correlation coefficients obtained in all the analytical matrices examined meet the acceptance criterion specified in Table IV. Additionally, the response plots show neither large nor 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 in all three extraction solvents.

Accuracy

Accuracy results, presented as the % spike recovery, are summarized in Table VI–VII. All the accuracy results met the acceptance criteria for this performance parameter.

Precision

Inter-run precision was assessed via six sequential injections of a standard and working sample containing approximately 60 ng/mL Tinuvin and 30 ng/mL T-RS. The inter-run experiment

was repeated in either two or four separate analytical runs, thus producing intra-run information.

The precision results are shown in Table IX. Inter-run precision was excellent, typically 5% RSD or less, and the intra-run precision was less than 9%. Both of these outcomes met the acceptance criterion.

Limit of quantitation (LOQ)

Calculated LOQ values (based on 10 times the S/N for the lowest concentration standards) were 0.13 ng/mL for Tinuvin 770 and 0.05 ng/mL for T-RS. The accuracy and precision data obtained for the lowest concentration test sample readily meet the acceptance criteria for these parameters, confirming that the practical or working LOQ is much less than the concentrations of the analytes in this sample (10 ng/mL for Tinuvin 770, 5 ng/mL for T-RS).

Sample and standard stability

Stability was addressed over the course of an analytical run lasting 26 h. The response 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 both analytes and all the sample matrices, which meets the acceptance criterion.

Validation summary

The chromatographic method met all the stated acceptance criteria and, therefore, is deemed to be valid for the purpose of quantitating the target analytes in aqueous extraction media.

System suitability

Method validation confirms that a properly implemented method will produce information of known and acceptable quality. Method validation does not, however, provide any assurance that a method has been 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 (S/N), response stability, and calibration curve linearity were performed in each of the four runs included in the validation exercise. The system suitability results (Table X) were used to establish acceptance criteria (Table X) that must be met in order for subsequent runs of this method to be deemed to be valid.

Extraction Study

The results of the extraction study are summarized in Table XI. The tested material contained readily measureable quantities of both Tinuvin and T-RS. Because the assay is based on MS–MS detection, the resultant chromatograms were free from interferences, and as expected no peaks were observed in the extraction blanks. The concentrations of these analytes in the extracts were highest in extraction media at the pH extremes.

Conclusion

An LC–MS–MS method has been developed for the purpose of quantitating Tinuvin 770 and a related substance at low concentrations [200 ng/mL (ppb) or less] in aqueous extracting media with a pH between 2 and 8. The developed method was validated for this application by assessing performance characteristics including accuracy, response linearity, precision, specificity, and solution stability. The validated method is suitable for the quan-

Table IX. Precision Results

Analyte	Solution Type	Inter-run Precision (% RSD, *n* = 6) Run 1	Run 2	Run 3	Run 4	Intra-run Precision % RSD
T-770	Water	2.6	4.5	–	–	8.1*
	pH 2	3.2	1.5	2.9	3.1	7.3†
	pH 8	5.1	1.8	4.3	2.7	6.9†
T-RS	Water	2.7	3.6	–	–	5.2*
	pH 2	1.3	0.47	2.4	5.1	5.6†
	pH 8	0.79	1.3	4.2	4.8	8.2†

* Data for two analytical runs, n = 12. † Data for four analytical runs, n = 24.

Table X. System Suitability Data and Adopted Acceptance Criteria

Run #	Tinuvin 770 Precision*	S-to-N†	Linearity (r)‡	Response Stability§	T-RS Precision*	S-to-N†	Linearity (r)‡	Response Stability§
1	1.4	188	0.9999	2.5	1.6	1730	1.000	0.55
2	3.1	478	0.9991	4.7	0.55	765	1.000	0.95
3	1.3	792	0.9995	8.9	4.6	965	0.9983	12.7
4	2.6	462	0.9992	8.3	4.5	491	0.9999	4.0
Criterion:	NMT 10%	≥ 100	NLT 0.9900	NMT 15%	NMT 10%	≥ 100	NLT 0.9900	NMT 15%

* %RSD of six sequential injections of an intermediate conc. standard made at the beginning of a run.
† S-to-N = signal to noise: Measured using the lowest conc. standard containing ~ 5 ng/mL T-RS and 10 ng/mL T-770.
‡ Obtained from duplicate injections of four calibration standards.
§ %RSD of all injections made of an intermediate concentration standard throughout the course of a run.

Table XI. Concentrations of the Target Extractables in the Test Material

Extraction Medium	Extractable amounts of the targets in the test material (µg/g) Tinuvin 770	T-RS
pH 2	1730	470
Water	1180	195
pH 8	1550	255

titation of these analytes in the concentration range of 1–200 ng/mL.

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