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Analysis of polycyclic aromatic hydrocarbons in metered dose inhaler drug formulations by isotope dilution gas chromatography/mass spectrometry

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Abstract: Organic compounds extracted into metered dose inhalers (MDIs) from the rubber components of the metering valve are of increasing interest in the development of these formulations. Polycyclic aromatic hydrocarbons (PAHs) are a class of extractable organic compounds whose source is the carbon black commonly used as a reinforcing agent in rubber. The analytical method for PAHs described in this report employs "cold filtration" to remove the suspended drug substance and excipients, and gas chromatography/mass spectrometry (GC/MS) for separation and detection of individual PAHs. After filtration, stable isotope labelled analogues of target PAHs are spiked into the drug product to act as internal standards, correcting for recovery (termed "isotope dilution GC/MS"). Validation of the method was accomplished with respect to linearity, precision, limit of detection/quantitation, selectivity and ruggedness. Application to a variety of MDI drug product formulations revealed that certain PAHs are present at the ng/inhaler level.

Keywords: *Metered dose inhaler; gas chromatography/mass spectrometry; polycyclic aromatic hydrocarbons; analytical method; method validation; isotope dilution.*

Introduction

Organic compounds which can be extracted into metered dose inhalers (MDIs) from the rubber components of the dose metering valve are of increasing interest in the development of these formulations [1]. These extractable organic compounds are of two types: those which are added purposefully to a rubber formulation and act as plasticizers, antioxidants, stabilizers, accelerators to the vulcanization process etc.; and those that are trace level contaminants either in the rubber itself or in one or more of the additives. An example of the latter type is the class of compounds known as the polycyclic aromatic hydrocarbons (PAHs) which are present at trace levels in carbon black, a reinforcing agent used in many rubber formulations. Some representative examples of PAHs are shown in Fig. 1. PAHs are formed during the incomplete combustion of organic matter (i.e. fossil fuels) [2] and occur widely in the environment at trace levels [2–4], particularly in the atmosphere where the levels generally parallel industrial and urban development [3].

The analysis of PAHs in MDIs requires a simple extraction procedure which can be applied to a variety of aerosol drug products and formulations, and an analytical technique capable of high sensitivity and compound specific detection. This report describes the development and validation of a quantitative analytical method for PAHs in MDI drug products which employs a "cold filtration" extraction procedure with subsequent separation and detection of individual target PAHs by combined gas chromatography/mass spectrometry (GC/MS). GC/MS is one of the most powerful trace organic analytical techniques available, with the capability of separating and individually detecting scores of organic compounds in a single sample. The capability of the mass spectrometer to distinguish stable isotopes also allows for the use of stable isotope labelled analogues of individual analytes as internal standards in quantitative assays, a technique referred to as "isotope

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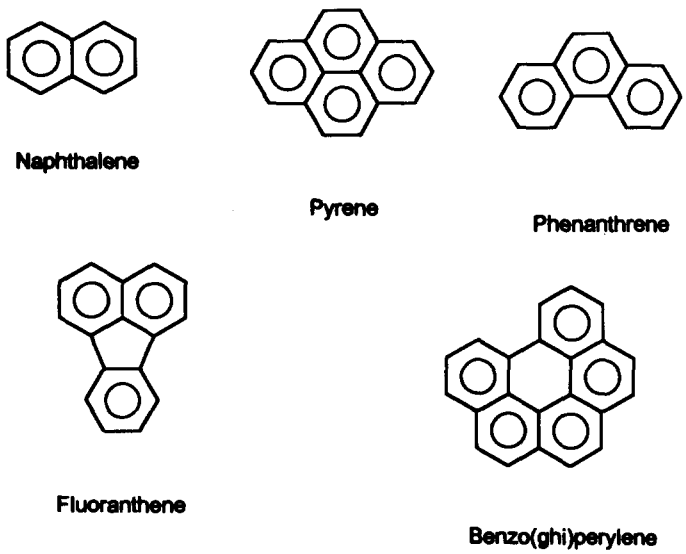

Figure 1
Examples of polycyclic aromatic hydrocarbons (PAHs): (1) naphthalene; (2) pyrene; (3) phenanthrene; (4) fluoranthene; (5) benzo(ghi)perylene.

dilution". These internal standards can significantly reduce systematic error (bias) from several sources including sample stability prior to analysis, analyte loss during both the extraction procedure and post-extraction sample workup, and from the calibration procedure. Isotope dilution GC/MS has been widely utilized in both the biomedical [5] and environmental fields [6].

As with any analytical method employed for pharmaceutical analysis, the isotope dilution GC/MS method for PAHs in MDIs required validation and the generation of "system suitability" criteria. Validation experiments performed for target PAHs in this study include linearity, precision, limit of detection/quantitation, selectivity and ruggedness. System suitability criteria which are applied prior to any analysis include tune and calibration of the mass spectrometer, chromatographic resolution, and sensitivity. Selected results from the validation and system suitability experiments are presented and discussed. The validated method has been applied over the past several years to a variety of MDI drug products and formulations, and some representative PAH quantitative profile results are presented in a variety of formats.

Materials and Methods

Reagents and materials

Unlabelled PAH standards (16 target PAHs) were obtained either as neat compounds (Kit 610-S) or in solution as prepackaged ampules (Kit 610-N) from Supelco, Inc. (Bellefonte, PA, USA). Samples in ampules should be used promptly after opening. Benzo(e)pyrene was obtained as a neat compound from Aldrich Chemical Company (Milwaukee, WI, USA). Deuterium labelled PAHs for use as internal standards were obtained from Cambridge Isotope Laboratories (Woburn, MA, USA) as neat compounds. A "cocktail" mixture of certain labelled PAHs at a concentration of 200 $\mu g\ ml^{-1}$ in d_2-dichloromethane/d_4-methanol is also available (Cambridge). Trichlorofluoromethane was obtained from Aldrich and was redistilled immediately prior to use to remove trace organic impurities. Other solvents used were Fisher Optima™ (Fisher Scientific, Springfield, NJ, USA), or Burdick & Jackson Brand High Purity Solvent (Muskegon, MI, USA).

Preparation of standard solution

A stock solution containing 10 $\mu g\ ml^{-1}$ of unlabelled target PAHs (seventeen individual target compounds) was prepared by dissolving accurately weighed quantities of each neat compound in toluene in a 250-ml volumetric flask. This stock solution was found to be stable and could be used for a period of up to 4 weeks when stored at 2°C. Deuterated internal standard stock solutions were prepared at 10 $\mu g\ ml^{-1}$ in a similar manner. Alternatively, stock solutions of both unlabelled and labelled PAHs could be prepared by appropriate

dilutions of ampulated PAH solutions. Calibration solutions were prepared by diluting increasing amounts of target PAHs and a constant amount of internal standard stock solution with toluene in volumetric flasks. The calibration solutions were analysed and calibration curves generated on each day of sample analysis.

Extraction procedure

The "cold filtration" extraction procedure is designed to separate the drug substance/excipient suspension from the propellant solution which contains the extractable organic compounds, including the target PAHs. A sample of 10 MDIs is cooled over solid dry ice for 10 min at which point the inhalers are individually opened with either a tubing cutter or a jeweler's saw. The contents of each MDI are then passed through a Whatman (Hillsboro, OR, USA) cellulose nitrate filter using a previously cooled Buchner flask and sinter funnel. After all 10 MDIs have been filtered, the residue on the filter is washed with three 15-ml aliquots of trichlorofluoromethane, which is collected in the same Buchner flask. (Note: the authors are well aware of the potential future problems with trichlorofluoromethane availability, which may necessitate the evaluation of an alternate solvent such as methylene chloride.) The filter and its contents are then discarded. A measured amount of internal standard solution (500 μl) is then added to the contents of the Buchner flask and the sample evaporated to dryness. Toluene (2 ml) is added to the Buchner flask to dissolve the analyte residue, and the resulting sample solution collected in a 5-ml volumetric flask. The Buchner flask is then washed with three 1 ml aliquots of toluene which are transferred to the same volumetric flask. After diluting to volume with toluene, the sample is ready for GC/MS analysis.

A sample blank was prepared on each day of analysis to demonstrate that all materials and glassware were free of interferences. Each blank consisted of three 15-ml aliquots of trichlorofluoromethane passed through the cellulose nitrate filter, internal standards added, and subsequently treated as a sample solution.

GC/MS instrumentation and conditions

GC/MS analyses were performed on a Hewlett–Packard 5970B Mass Selective Detector (MSD) interfaced with a 5890 Series II gas chromatograph and under the control of an HP-UX MS ChemStation data system (Hewlett–Packard Company, Palo Alto, CA, USA). A J&W DB-5 capillary column (J&W Scientific, Folsom, CA, USA) of dimensions 30 m × 0.25 mm with a 0.25-μm film thickness was employed with the following helium carrier gas flows (at 10 lb in^{-2} column head pressure): column flow 1 ml min^{-1}, split vent 50 ml min^{-1}, septum purge vent 2 ml min^{-1}. Splitless injections of 1 μl of toluene solution (either standards or samples) were accomplished with a Hewlett–Packard 7673 autosampler/injector at an injection port temperature and MSD transfer line temperature of 300°C. The injector purge was turned off for a period of 0.6 min during the injection process (splitless injection). For elution of target PAHs the following temperature program was used: initial temperature 110°C, initial time 1 min, rate 10°C min^{-1}, final temperature 300°C, final time 10 min. The mass spectrometer was operated in electron ionization (EI) mode with selected ion monitoring (SIM) employed for high sensitivity data acquisition. SIM retention windows were set up around the known elution times of individual target PAHs and internal standards for monitoring of the appropriate molecular ions ($M^{+\cdot}$). The dwell time for individual channels (monitored ions) was 20 ms. The mass spectrometer was tuned and the mass scale calibrated according to criteria established by the manufacturer with perfluorotributylamine (heptacosa; FC43) under the control of the HP data system.

Treatment of data

Calibration curves are created automatically by the HP-UX data system software package and consist of integrated peak area ratios (peak area analyte/peak area internal standard) vs the corresponding concentration ratios with an appropriate linear regression. The data system also computes individual PAH levels in unknown samples based on the appropriate calibration curve, integrated peak areas, and the known amount of internal standard spike. Results are reported in units of μg ml^{-1} and are then converted to μg/inhaler for ease of comparison. Due to the significant mass difference between analytes and the deuterated internal standards, it is not necessary to correct the peak area ratios for isotope overlap before plotting and regression analysis [6, 7].

Results and Discussion

Basis of the SIM method

The target PAHs included in this analytical method are listed in Table 1, along with their corresponding deuterated internal standard(s) and monitored ions. Note that in all cases, the molecular ion ($M^{+\cdot}$) of each target PAH and internal standard was chosen for monitoring by the mass spectrometer. Figure 2 shows the complete EI mass spectra of pyrene and its internal standard d_{10}-pyrene, which are representative of PAH mass spectra. The majority of the ion current in both cases is carried by the molecular ion (singly charged) and by the doubly charged ion (M^{+2}), demonstrating that maximum sensitivity in a quantitative assay would be achieved by monitoring the molecular ions. In these mass spectra there is little fragmentation as the polyaromatic structure lends significant stability to both the singly and doubly charged molecular ions. A typical GC/MS analysis demonstrating the separation of target compounds is shown in Fig. 3. Target PAHs are identified by comparing the retention times of the characteristic molecular ion peaks with those of the authentic standards. Although not a component of this method, it is possible to simultaneously monitor the doubly charged ions which provides an additional verification that the appropriate target compounds are being detected and quantified. Isotope overlap is not a consideration in this analysis because there is significant mass separation between analyte and internal standard ions (10 mass units in the case of pyrene).

Method validation

The validation criteria chosen for the analytical method included linearity, precision, limit of detection/quantitation, selectivity and ruggedness, as suggested by the USP General Test Chapter 1225 [8] for the determination of impurities and degradation products in bulk drug substances or in finished pharmacological products [also see 9–11].

The linearity criteria for target PAHs was assessed in two ways, first as linearity of the mass spectrometer's response and then as linerity of recovery of both analyte and internal standard from the appropriate drug product matrix. Linearity of instrument response was determined by injecting five standard solutions containing each PAH over the appropriate concentration range (for example 0.05–2.5 μg/inhaler) for the particular drug product and a constant level of each deuterated internal standard. SIM peak area measurements were used to calculate peak area ratios (area PAH/area d-PAH) and these were plotted vs concentration ratio ([PAH]/[d-PAH]). Linearity of recovery was determined by spiking an appropriate drug product matrix with PAH standard solutions over the same concentration range and subjecting these spiked solutions to the same extraction and analysis procedure as the MDIs. The drug product matrix included the drug substance, excipients and other ingredients in the same proportions as in the MDI formulation. Linearity of recovery is demonstrated by comparison of the slope, *y*-intercept, and correlation coefficient from this plot with those from the appropriate instru-

Table 1
Target PAHs and their corresponding deuterated internal standards

PAH	Monitored ion	Internal standard	Monitored ion
Naphthalene	128	d_8-naphthalene	136
Acenaphthylene	152	d_8-acenaphthylene	160
Acenaphthene	154	d_8-acenaphthylene	162
Fluorene	166	d_{10}-fluorene	176
Phenanthrene	178	d_{10}-phenanhrene or d_{10}-anthracene	188
Anthracene	178	d_{10}-phenanthrene or d_{10}-anthracene	188
Fluoranthene	202	d_{10}-fluoranthene	212
Pyrene	202	d_{10}-pyrene	212
Benzo(a)anthracene	228	d_{12}-chrysene or d_{12}-benzo(a)anthracene	240
Chrysene	228	d_{12}-chrysene or d_{12}-benzo(a)anthracene	240
Benzo(b)fluoranthene	252	d_{12}-benzo(b)fluoranthene or d_{12}-benzo(k)fluoranthene	264
Benzo(k)fluoranthene	252	d_{12}-benzo(b)fluoranthene or d_{12}-benzo(k)fluoranthene	264
Benzo(e)pyrene	252	d_{12}-benzo(a)pyrene or d_{12}-benzo(e)pyrene	264
Benzo(a)pyrene	252	d_{12}-benzo(a)pyrene or d_{12}-benzo(e)pyrene	264
Indeno(123-cd)pyrene	276	d_{12}-dibenzo(ah)anthracene	292
Dibenzo(ah)anthracene	278	d_{14}-dibenzo(ah)anthracene	292
Benzo(ghi)perylene	276	d_{12}-benzo(ghi)perylene	288

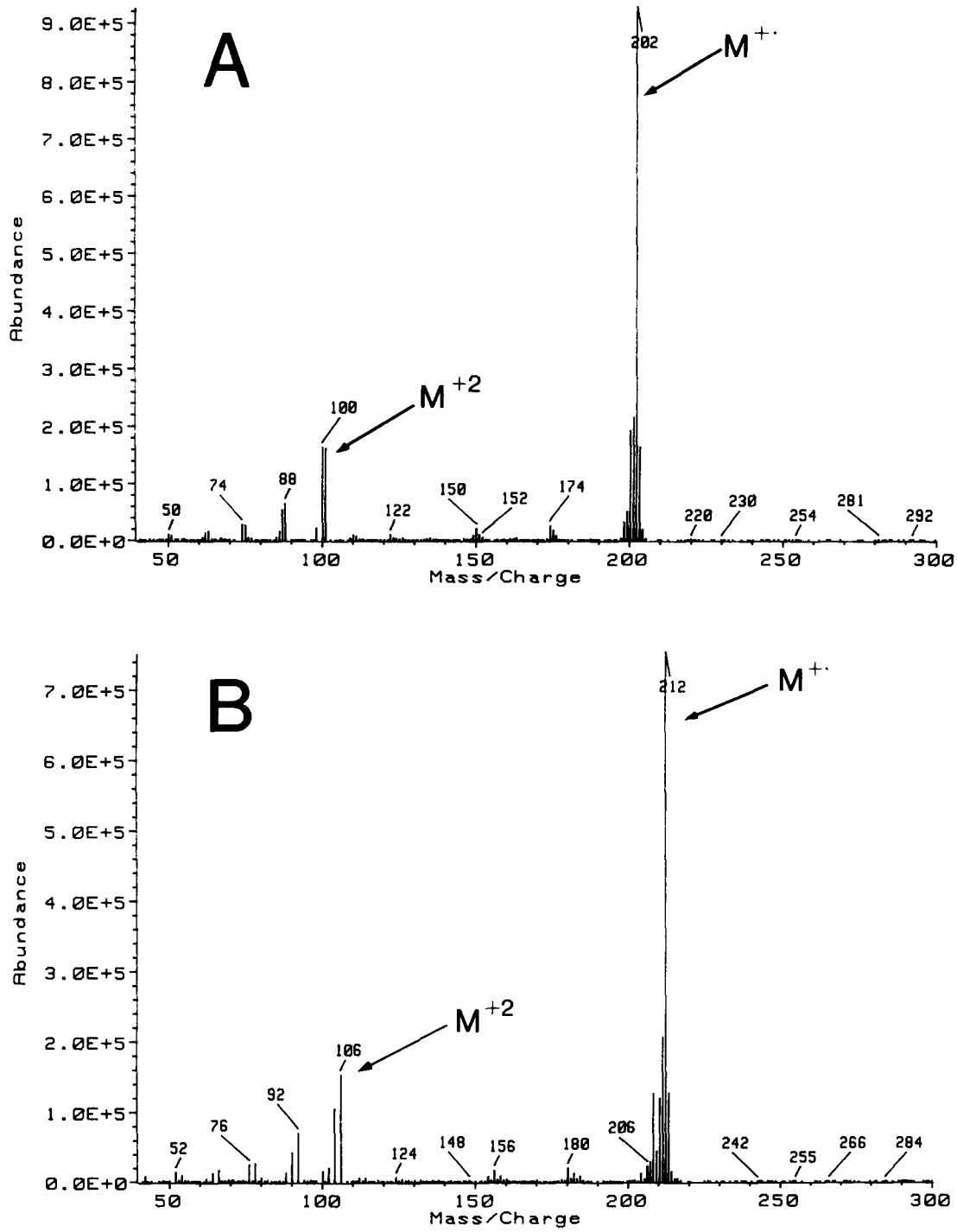

Figure 2
Electron ionization (EI) mass spectra of (A) pyrene, and (B) D_{10}-pyrene. Note the molecular ions ($M^{+\cdot}$) and doubly charged ions (M^{+2}).

ment response linearity experiment. Representative linearity and linearity of recovery results for a particular drug product assay are shown in Table 2. Note that in all cases, correlation coefficients are >0.999. It is important to emphasize that linearity of recovery is drug product and formulation specific and must be demonstrated for each target PAH in all cases.

Precision of the method for a particular drug product was assessed by analysing five MDI samples from the same production batch of MDIs, all retained under identical storage

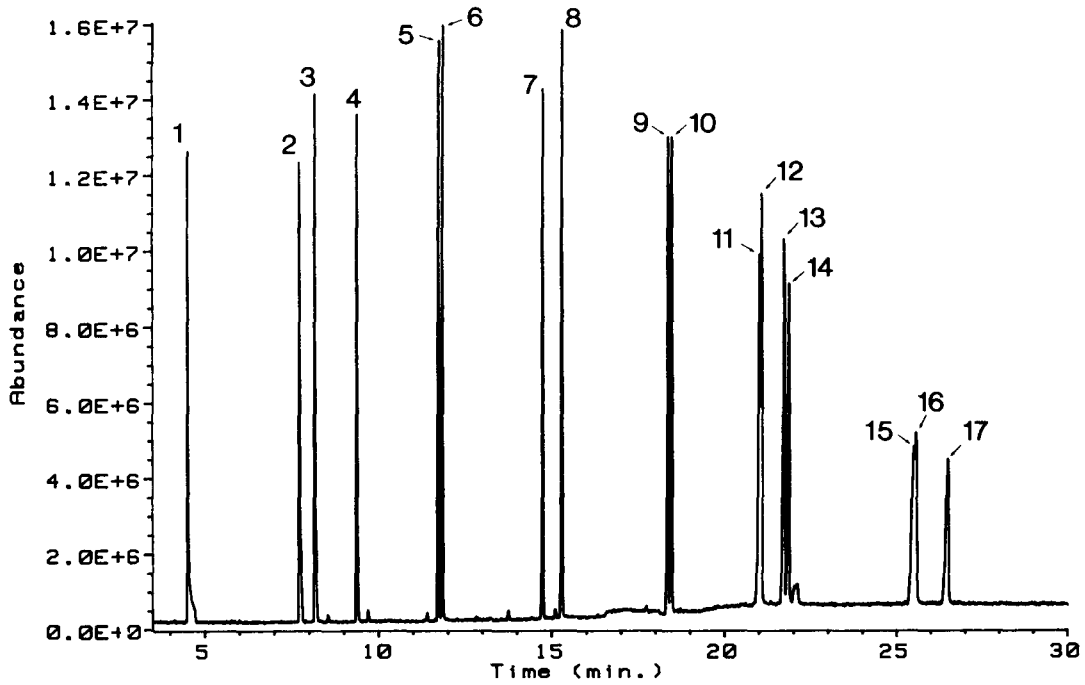

Figure 3
Reconstructed total ion chromatogram demonstrating the separation of target polycyclic aromatic hydrocarbons. This chromatogram was derived from scanning mass spectrometric data. (1) naphthalene; (2) acenaphthylene; (3) acenaphthene; (4) fluorene; (5) phenanthrene; (6) anthracene; (7) fluoranthene; (8) pyrene; (9) benzo(a)anthracene; (10) chrysene; (11) benzo(b)fluoranthene; (12) benzo(k)fluoranthene; (13) benzo(e)pyrene; (14) benzo(a)pyrene; (15) indeno(123-cd)pyrene; (16) dibenzo(ah)anthracene; (17) benzo(ghi)perylene.

Table 2
Representative linearity and linearity of recovery results for a drug product assay

PAH	Slope	Intercept	Correlation coefficient
Naphthalene	0.973*	0.045	0.9998
	0.993†	0.033	0.9998
Acenaphthene	0.658	0.021	0.9998
	0.663	0.014	0.9992
Phenanthrene	1.059	0.067	0.9997
	1.061	0.080	0.9990
Fluoranthene	1.070	0.059	0.9997
	1.085	0.069	0.9996
Pyrene	1.034	0.059	0.9998
	1.040	0.072	0.9997
Benzo(e)pyrene	1.485	0.056	0.9998
	1.487	0.087	0.9994
Benzo(a)pyrene	0.827	0.032	0.9996
	0.853	0.054	0.9997
Benzo(ghi)perylene	1.320	−0.016	0.9996
	1.350	−0.028	0.9998

*Top value is linearity.
†Bottom value is linearity of recovery.

conditions. Some typical results are shown in Table 3, including the average content for the 10 target PAHs detected in this particular product batch, confidence limits and relative standard deviations. Note that the analytical precision varies from 4 to 33% and is directly correlated with analyte concentration. Lower precision was observed for those PAHs present at or near the limit of quantitation (i.e. benzo(ghi)perylene). In general, a precision of ≤10% RSD was observed for PAHs determined near the middle of the calibration range.

The detection limit is defined as that concentration of an individual PAH that produces a

Table 3
Precision of PAH determination in a typical MDI drug product

PAH	PAH content (μg/inhaler) Sample 1	2	3	4	5	Average	% RSD
Naphthalene	0.25	0.26	0.23	0.27	0.25	0.25 ± 0.01	4.0
Acenaphthylene	0.34	0.35	0.35	0.40	0.37	0.36 ± 0.03	8.3
Fluorene	0.03	0.03	0.02	0.03	0.02	0.03 ± 0.01	33
Phenanthrene	1.28	1.33	1.63	1.51	1.46	1.44 ± 0.14	9.7
Anthracene	0.09	0.09	0.11	0.10	0.10	0.10 ± 0.01	10
Fluoranthene	0.90	0.95	1.27	0.99	1.05	1.03 ± 0.14	14
Pyrene	0.99	1.04	1.29	1.10	1.15	1.11 ± 0.12	11
Benzo(e)pyrene	0.06	0.04	0.05	0.06	0.04	0.05 ± 0.01	20
Benzo(a)pyrene	0.03	0.02	0.03	0.03	0.02	0.03 ± 0.01	33
Benzo(ghi)perylene	0.04	0.03	0.05	0.06	0.05	0.05 ± 0.01	20

Table 4
Limit of detection/quantitation results for selected target PAHs

PAH	Limit of detection* (ng/inhaler)	Limit of quantitation† (ng/inhaler)
Naphthalene	0.7	4
Acenaphthylene	0.7	4
Fluorene	0.9	5
Phenanthrene	0.9	5
Fluoranthene	0.7	4
Pyrene	0.7	4
Benzo(ghi)perylene	6.0	30

*Rounded to one decimal place.
†Five times the rounded limit of detection.

response three times that of the average noise level in the chromatographic baseline (signal-to-noise = 3). Method detection limits were determined by extracting and analysing drug product matrix solutions spiked with approximately 15 ng ml^{-1} of each PAH to be validated. The detection limit was estimated by injecting the test solution, determining the signal-to-noise ratio at this concentration, and extrapolating to the appropriate level

$$\text{Limit of detection (ng/inhaler)} = (C_a N_{rms}/S) \times 3/10, \quad (1)$$

where C_a = concentration of PAH in calibration solution (ng ml^{-1}), S = signal measured from the mid-point of the peak-to-peak noise to the maximum of the signal peak, N_{rms} = (1/5) × peak-to-peak noise, 3 = ratio of signal-to-noise required for limit of detection and 10 = number of inhalers. The limit of quantitation is defined as five times the limit of detection. Representative results are shown in Table 4.

The selectivity for individual target PAHs, in a particular drug product was demonstrated by extracting and analyzing a solution of appropriate drug product matrix containing no target PAHs or internal standards. Selected ion chromatograms for each monitored ion (PAH and d-PAH molecular ions) were generated and examined for interferences at the appropriate retention times. Figure 4 shows two such chromatograms covering the retention times for pyrene and its deuterated analogue, both of which exhibit no interferences. These results were typical for all target PAHs across a range of MDI drug products examined. An alternative way to assess selectivity is to compare results from the analysis of spiked drug product matrix solutions with those of a standard solution containing target PAHs and internal standards present at the same level as the spike. Typical results indicated that the average bias between the standard and spiked samples was approximately 0.6% when target PAHs were spiked at 1 μg ml^{-1} (internal standards spiked at the same concentration).

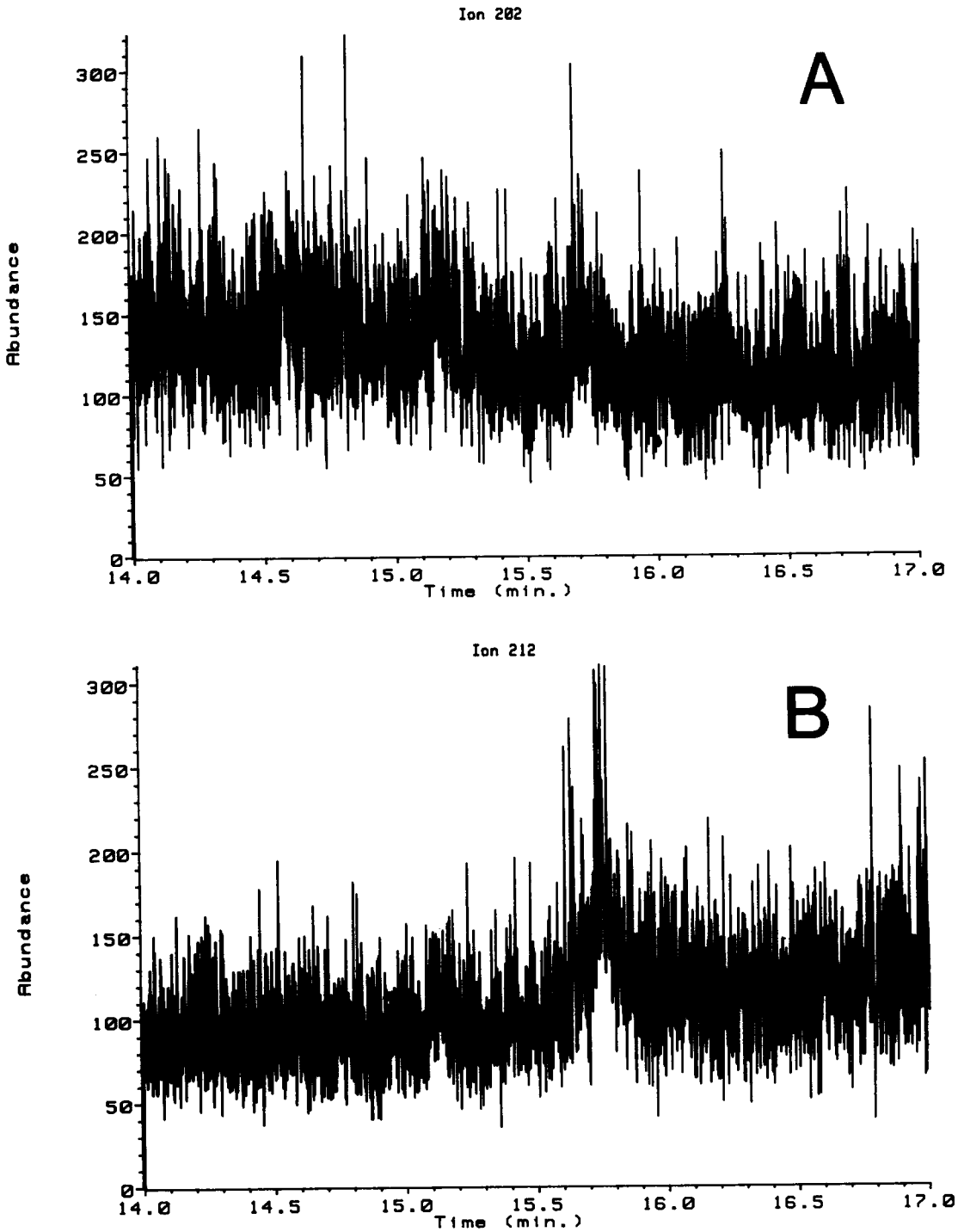

Figure 4
Example selectivity experiment for pyrene: (A) single ion chromatogram for *m/z* 202 (pyrene); and (B) single ion chromatogram for *m/z* 212 (d_{10}-pyrene). Note the lack of interferences in either channel.

Ruggedness is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal conditions. During the course of this study the effect of different analysts, different instruments and different days of conducting analyses were assessed. Table 5 presents some typical results for the analysis of an MDI drug product batch by two different analysts on different days with the same GC/MS system. The percent difference in results between the two analysts is clearly a function of analyte

Table 5
Typical ruggedness results for PAH determination in an MDI drug product batch employing different analysts on different days with the same instrument

PAH	Analyst no. 1 (μg/inhaler)	Analyst no. 2 (μg/inhaler)	% difference
Naphthalene	0.36	0.28	28.6
Acenaphthylene	0.52	0.52	0.0
Acenaphthene	NT*	<0.05	—
Fluorene	<0.05	<0.05	—
Phenanthrene	2.19	2.57	17.4
Anthracene	0.16	0.17	6.3
Fluoranthene	1.38	1.51	9.4
Pyrene	1.58	1.58	0.0
Benzo(a)anthracene	ND†	ND	—
Chrysene	ND	ND	—
Benzo(b)fluoranthene	ND	ND	—
Benzo(k)fluoranthene	ND	ND	—
Benzo(e)pyrene	<0.05	<0.05	—
Benzo(a)pyrene	<0.05	<0.05	—
Dibenzo(ah)anthracene	ND	ND	—
Indeno(123-cd)pyrene	ND	ND	—
Benzo(ghi)perylene	0.07	<0.05	40.0
Total	6.41	6.88	7.3

*NT — not tested.
†ND — not detected.

concentration and in this way parallels the precision results. For target PAHs near the middle of the calibration range (fluoranthene and pyrene, for example) the percent difference is low (<10%). As the analyte level nears the estimated quantitation limit the bias predictably increases (note that levels above the limit of quantitation but below the lowest point on the calibration curve are reporated as <0.05, which is the lowest calibration level). This bias could in theory be reduced by having different calibration ranges for each target PAH in a given MDI drug product which placed each analyte level near the middle of the appropriate calibration range. For most quantitative MDI studies this is neither practical nor desirable since high accuracy near the quantitation limits is not required.

System suitability criteria

In addition to the daily calibration curves which must be linear and the blank analysis which must be free of interferences, certain other criteria must be met in order to ensure that accurate results are obtained. These additional elements which include chromatographic resolution and sensitivity are referred to as "System Suitability Criteria", and are determined from the benzo(e)pyrene and benzo(a)pyrene peaks in the lowest calibration solution. Sensitivity is verified by estimating the limit of quantitation for both analytes according to the previously defined equation. The calculated quantitation limits should be ≤10 ng per inhaler for both PAHs. The chromatographic resolution between the benzo(e)pyrene and benzo(a)pyrene peaks in the lowest calibration solution must be ≥0.8 using the calculation procedure defined in USP XXII [12], and presented in Fig. 5. Sample analyses may only proceed after the mass spectrometer has been tuned and the mass scale calibrated, linear calibration curves have been generated, the system suitability criteria have been met, and the blank solution has been shown to be free of interferences.

Representative PAH profiles

An example of data generated by the validated GC/MS method is shown in Table 5 (the "ruggedness" experiment). Other examples from various MDI drug products and storage conditions are presented in Table 6. These results are generally representative of those generated over the course of several years of sample analyses with a wide variety of MDI drug products and formulations. Although this tabular form of data presentation has obvious advantages (for the development of specifications, for example), an alternative presentation format has been investigated and demonstrated to be both

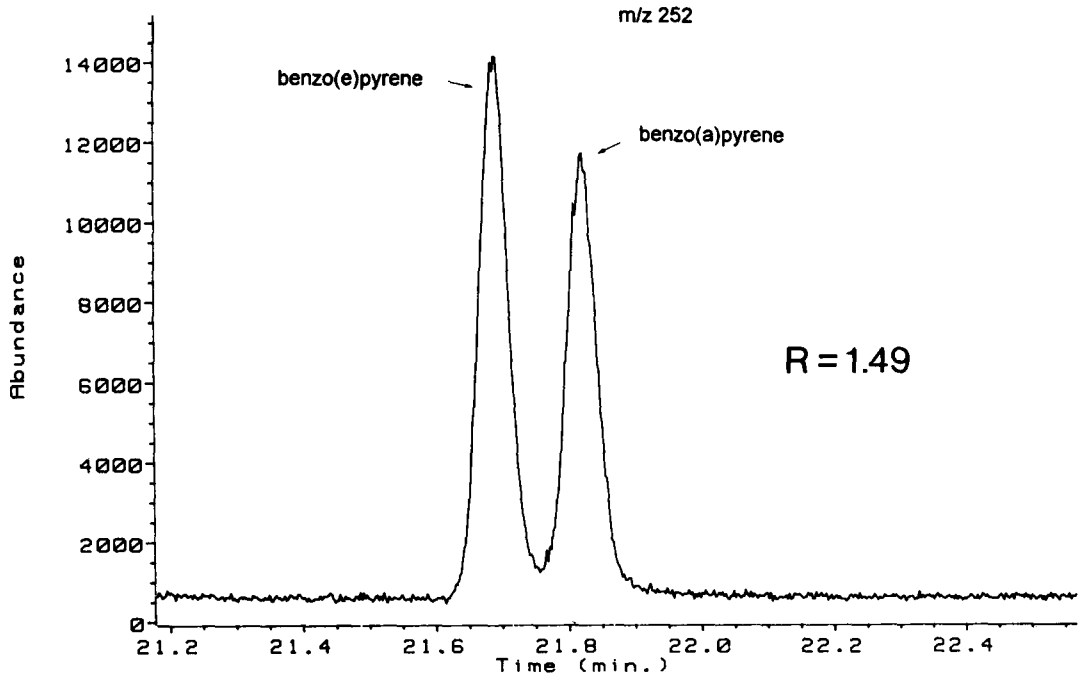

Figure 5
Example of the chromatographic resolution system suitability criterion based on the benzo(e)pyrene and benzo(a)pyrene peaks in the analysis of the lowest calibration solution.

Table 6
PAH quantitative profiles in various MDI drug products

PAH	Product A (μg/inhaler)	Product B (μg/inhaler)	Product C (μg/inhaler)
Naphthalene	0.29	0.15	0.57
Acenaphthylene	0.43	0.22	0.45
Acenaphthene	ND*	ND	ND
Fluorene	<0.05	ND	<0.05
Phenanthrene	1.96	0.88	2.14
Anthracene	0.10	ND	0.12
Fluoranthene	1.20	0.53	1.37
Pyrene	1.26	0.61	2.13
Benzo(a)anthracene	ND	ND	ND
Chrysene	ND	ND	ND
Benzo(b)fluoranthene	ND	ND	ND
Benzo(k)fluoranthene	ND	ND	ND
Benzo(e)pyrene	0.08	<0.025	0.08
Benzo(a)pyrene	<0.05	ND	<0.05
Dibenzo(ah)anthracene	ND	ND	ND
Indeno(123-cd)pyrene	ND	ND	ND
Benzo(ghi)perylene	0.08	0.03	0.06
Total	5.50	2.45	7.02

*ND — not detected.

complementary to the tabular form and to provide a different perspective on the PAH profiles.

The term "profile" is defined as a pictorial or graphical representation of data. Such a representation of the quantitative PAH data can be derived by combining the selected ion current chromatograms from the PAH molecular ions (excluding the internal standard ion chromatograms) into a single chromatogram which is normalized to the height of the largest peak. This data manipulation exercise is accomplished by the HP ChemStation datasystem via a macro program which was devel-

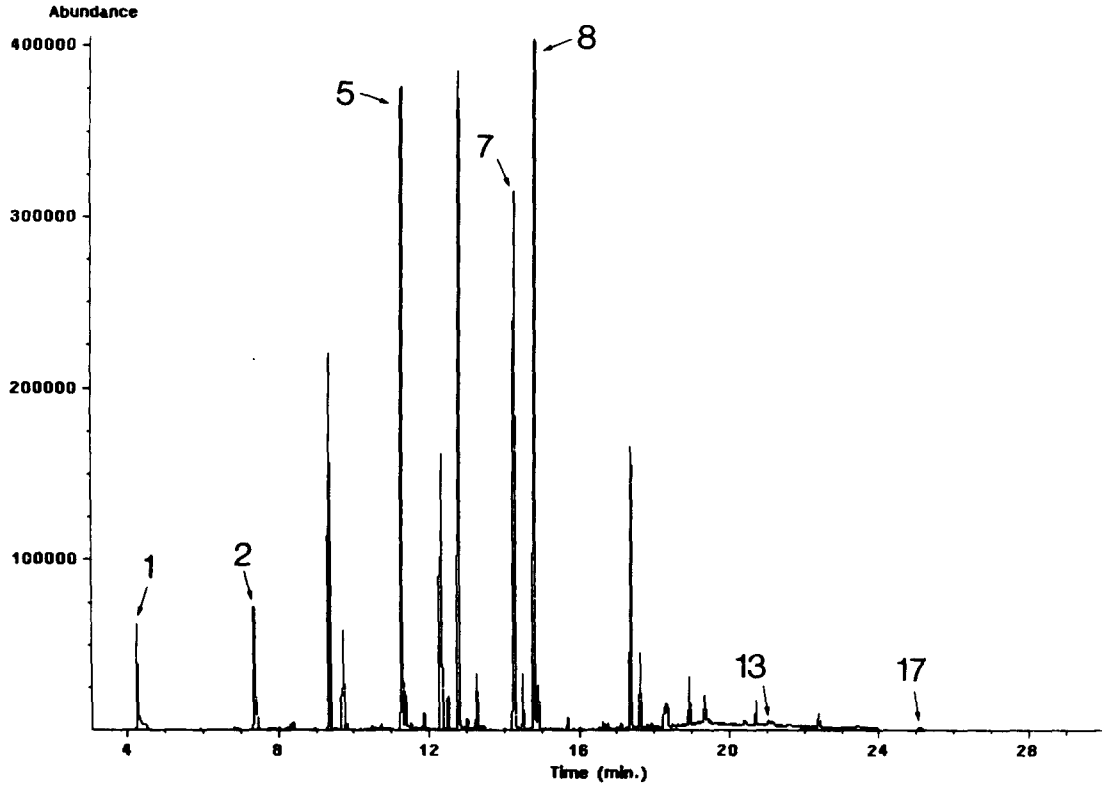

Figure 6
Polycyclic aromatic hydrocarbon (PAH) "profile" derived from the quantitative PAH data for Product B presented in Table 6. This plot is a computer constructed chromatogram derived by summing the single ion chromatograms for the monitored ions for each target PAH (excluding the internal standards) and normalizing relative to the peak height of the most abundant PAH detected. (1) naphthalene; (2) acenaphthylene; (3) acenaphthene; (4) fluorene; (5) phenanthrene; (6) anthracene; (7) fluoranthene; (8) pyrene; (9) benzo(a)anthracene; (10) chrysene; (11) benzo(b)fluoranthene; (12) benzo(k)fluoranthene; (13) benzo(e)pyrene; (14) benzo(a)pyrene; (15) indeno(123-cd)pyrene; (16) dibenzo(ah)anthracene; (17) benzo(ghi)perylene.

oped during the course of this study. A representative "profile" is shown in Fig. 6, which was created from the data presented in Table 6, Product B (note the expected approximate location for each target PAH from the total ion chromatogram in Fig. 3. The total ion chromatogram is derived from scanning data and not from selected ion current chromatograms). The additional peaks present in this chromatogram, besides those representing the target PAHs, result from other sample components eluting at different retention times from the target PAHs that also have ions in their mass spectra at the same nominal mass as a particular monitored ion and are detected in a retention window.

Conclusions

A quantitative analytical method was developed and validated for target polycyclic aromatic hydrocarbons present in metered dose inhaler drug formulations. The method employs a "cold filtration" extraction procedure combined with the well established technique of isotope dilution gas chromatography/mass spectrometry, and was demonstrated to be highly sensitive and specific for target PAHs, as well as rugged and capable of being applied to a variety of MDI drug products. Data indicate that certain PAHs are indeed present in MDI drug products, but at very low levels (ng/inhaler) relative to the drug substance.

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