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Quantitation of acetol in common pharmaceutical excipients using LC–MS

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Abstract

A method for the quantification of acetol at μg/L levels in propylene glycol and glycerol, two common pharmaceutical excipients, was developed and validated. This simple yet highly specific method makes use of derivatization by O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) in aqueous solution at room temperature followed by analysis via LC–MS without sample pre-concentration, extraction, or cleanup. Kinetic studies indicated that the derivatization reaction was complete after 4.5 h. Preliminary investigations demonstrate the applicability of this method to the separation and identification of other electrophilic impurities. This suggests the potential for a simple, quantitative assay at room temperature in aqueous solution for the determination of a variety of electrophilic impurities in pharmaceutical excipients, without the need for sample concentration or extraction.

Keywords: Acetol; Derivatization; Pentafluorobenzylhydroxylamine hydrochloride; Excipients; Electrophilic impurities

1. Introduction

Electrophilic impurities and contaminants are a concern in many industries, and a great deal of research has been undertaken to develop methods capable of identifying and quantifying such compounds in everything from self-tanning creams [1] to human serum [2]. Much of this work has focused on detecting molecular markers of spoilage and other contaminants in foods, beverages, and drinking water [3–11]. Methods have also been developed to measure the electrophilic burden in ambient air [12–14], precipitation and ice cores [15–18], and swimming pool water [19,20] in order to address environmental and public health concerns.

Recently, there has been some interest in developing similar methods for the determination of electrophilic impurities in pharmaceutical excipients [21–23]. Such impurities may react with the active pharmaceutical ingredient (API) to form adducts and other drug impurities that may reduce drug efficacy or cause unwanted side-effects. This is particularly problematic with the development of highly potent APIs that make up only a small proportion of the final drug formulation. As a result, even trace amounts of unwanted electrophilic impurities in excipients may cause unacceptable levels of drug product impurities. Propylene glycol and glycerol are two examples of commonly used pharmaceutical excipients that are known to contain acetol as an electrophilic impurity.

Most methods developed to detect electrophilic impurities have made use of derivatization in order to improve recovery, peak shape, separation, and detection. The most commonly used derivative in HPLC studies is 2,4-dinitrophenylhydrazine (DNPH) [24], while O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) is the reagent of choice for many GC applications [25]. PFBHA reacts with electrophilic compounds to form the corresponding oxime in either aqueous or organic solution under relatively mild conditions. After reaction in an aqueous solution, products are usually extracted into an organic solvent prior to analysis, although pre-concentration methodologies via SPE have also been reported in a number of recent studies in order to increase sensitivity [3,8,15].

Although PFBHA is used most often for GC applications, there is evidence that it is also applicable to HPLC analysis [9,25]. This separation method has clear advantages over

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GC, as it may be used to analyze non-volatile or heat-labile compounds. This would allow one to monitor drug product and non-volatile impurities alongside volatile compounds. Additionally, some compounds cannot be analyzed via GC due to matrix interference effects [26]. The high heat of headspace conditions may also cause some excipients to degrade, potentially to electrophilic compounds, which would prevent accurate quantification of impurities. In addition, analysis via HPLC can be performed more quickly than headspace GC, which may require lengthy vial equilibration times. Finally, analysis of PFBHA derivatives is incompatible with FID technology, since the highly electronegative fluorine moieties greatly reduce the detector response [20]. Thus, GC–ECD or GC–MS systems are required for analysis.

Most HPLC methods have relied on UV–vis or fluorometric detection schemes, although some recent studies have also used mass spectrometry in order to gain more structural information on the derivatization products [13–15,23]. We have developed a method that makes use of the greater sensitivity of MS analysis coupled with the versatility and reliability of HPLC in order to quantify the levels of electrophilic impurities in common pharmaceutical excipients. This method was used to determine the concentration of acetol contamination in commercial samples of propylene glycol and glycerol. Sample preparation is greatly simplified as compared to similar methods, since analysis is performed on the reaction mixture itself without an additional organic extraction step. Good sensitivity is achieved without the need for sample pre-concentration.

2. Experimental

2.1. Chemicals and samples

Acetol (1-hydroxy-2-propanone), propylene glycol, aldehydes and ketones, and O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine were obtained from Sigma–Aldrich (Oakville, ON). Glycerol and acetonitrile (HPLC grade) were obtained from EMD (NJ, USA). Water was purified using a Milli-Q Gradient A10 system (Millipore, Cambridge, ON).

2.2. Standard and reagent solutions

A series of acetol standards, ranging in concentration from approximately 128 μg/L to 3200 μg/L, were prepared by diluting acetol in acetonitrile–water (5:95, v/v). A working solution of O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA, 2 mg/mL) was also prepared in acetonitrile–water (5:95, v/v).

2.3. Apparatus and chromatographic conditions

HPLC analyses were carried out on an Agilent (Mississauga, ON) 1100 series liquid chromatograph with UV–vis detection using a Waters (Mississauga, ON) Xterra Phenyl column (2.1 mm × 100 mm; 3.5 μm particle size) at ambient temperature. A flow rate of 0.5 mL/min was used with a sample injection volume of 50 μL. Mobile phase A consisted of acetonitrile–water (5:95, v/v) while mobile phase B consisted of acetonitrile alone. The gradient varied linearly from 0% to 100% B in 6 min and was held at 100% B until 7 min. A linear gradient to 0% B occurred until 7.1 min, and this was held until 9 min.

Mass spectral analyses were carried out on an LCQ Deca ion trap mass spectrometer (Thermo Electron, ME, USA) with an APCI ionization source in the positive mode. A parent ion of $m/z = 270 \pm 0.5$ was monitored and underwent collision induced decay (CID) to generate MS/MS spectra. The fragment ion at $m/z = 252$ was monitored to construct selected ion chromatograms. The mass spectrometer was set with a source heater temperature of 250 °C; a capillary temperature of 230 °C and voltage of 22.00 V; sheath and auxiliary gas flow rates of 20 and 56, respectively; a tube lens offset of −20.00 V; and an APCI source current of 5.00 μA.

Charged aerosol detection (CAD) analysis was performed with a Corona CAD from ESA Biosciences (MA, USA).

2.4. Time-course of the derivative formation

10.0 mL each of a 500 μg/L acetol solution and a 10 mg/mL PFBHA solution (molar ratio approximately 1:6900), both in acetonitrile–water (5:95, v/v), were combined and an aliquot was transferred to an HPLC vial and shaken. The final acetol concentration was 250 μg/L. Injections were made from this reaction vial at regular intervals over 6 h, and analyzed via LC–MS using the parameters outlined above. Similar experiments were conducted with propylene glycol samples.

2.5. Sample preparation and derivatization procedure

Propylene glycol and glycerol samples were diluted in acetonitrile–water (5:95, v/v). Dilution factors of 1/5–1/100 were used, depending on the sample. Two millilitres of each acetol standard and sample solution was added, individually, to 20 mL scintillation vials containing 2 mL of PFBHA working solution each. The mixture was allowed to stir at room temperature. Each reaction mixture was then transferred to vials for HPLC analysis.

2.6. Quantitative analysis

The five standard acetol solutions were treated according to the PFBHA derivatization reaction outlined above, resulting in standard solutions of acetol–PFBHA oxime with final concentrations ranging from 64 μg/L to 1600 μg/L of acetol. A line of best fit was constructed based on automated integration of peaks in the MS/MS selected ion chromatograms, with a correlation coefficient of 0.9944. Similar results were found based on manual integration of peaks in UV chromatograms monitoring $\lambda = 228$ nm.

3. Results and discussion

3.1. Development of derivatization reaction

A schematic representation of the acetol–PFBHA derivatization reaction is shown in Fig. 1. Sample preparation was

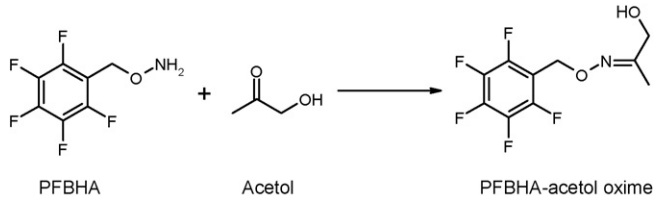

Fig. 1. Schematic representation of the acetol–PFBHA derivatization reaction.

optimized by performing the derivatization, with PFBHA, of acetol at approximately 100 mg/L, followed by dilution to a final assay concentration of approximately 50 μg/L. This was performed in the absence of either propylene glycol or glycerol. These results were compared to those obtained by performing the derivatization at 50 μg/L directly. The average normalized response (peak area per mg of acetol) was 36% (UV) or 64% (MS) higher when derivatization was performed on diluted samples, as opposed to stock samples diluted after derivatization. These data indicate that derivatization is less efficient at higher acetol concentrations. Due to this inefficiency, it is recommended that assay acetol concentrations be targeted below 1000 μg/L. All subsequent experiments described herein were performed in this assay range, and in all cases the molar ratio of PFBHA to acetol in derivatization reactions was 10:1 or higher.

3.2. Chromatographic method development

As a starting point for HPLC method development, a standard C18 reverse phase column was employed. The resulting chromatography, however, was not satisfactory and showed very poor peak resolution. It was anticipated that the aromaticity of the derivatization agent could be exploited through the use of a column with phenyl functionality. An Xterra phenyl column was found to provide adequate peak resolution and required minimal further method development. Organic and pH mobile phase modifiers and gradient times were optimized using this column, resulting in the current method.

Glycerol and propylene glycol do not show substantial absorbance in either the UV or visible spectra, and their low molecular weight greatly complicates detection by MS analysis.

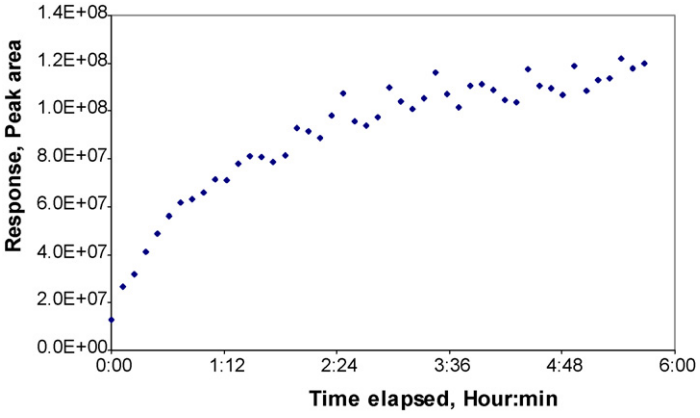

Fig. 2. Time-course of the acetol–PFBHA derivatization reaction. Conditions described in the text.

HPLC analysis using a CAD was thus performed to determine the retention times for glycerol and propylene glycol, which were found to elute in the solvent front at approximately 0.8–0.9 min. In order to prevent unnecessary contamination of the mass spectrometer and subsequently improve accuracy and precision, it is advised that the first 2.5 min of each run be diverted to waste. The diversion of high amounts of excipient from the MS source ensures the stability of the MS signal over the course of the analyses.

The PFBHA–acetol derivative was found to have a retention time of 5.66 min. The most abundant fragment ion in the MS/MS spectrum had $m/z = 251.93$, which most likely represents a loss of water from the parent ion of $m/z = 270$.

3.3. Time-course studies on the derivatization of acetol by PFBHA

A plot from the derivatization reaction time-course studies is shown in Fig. 2. This reaction appears to reach completion after approximately 4.5 h at room temperature, as evidenced by the plateau in the kinetic plot. Similar results were observed with acetol standards, both in acetonitrile–water (5:95, v/v) and in the presence of propylene glycol. The rate of reaction does not appear to be affected by the presence of excipient.

3.4. Method validation

The linearity of the method was validated using four standards, in triplicate, with acetol concentrations in the range from 64.6 μg/L to 969 μg/L. A linear relationship was found between the integrated peak area in the SIM mass chromatogram and the concentration of acetol standard, with a correlation coefficient of 0.9944. Not surprisingly, a linear relationship was also found between integrated peak area and concentration of acetol standard in the UV chromatogram, with a correlation coefficient of 0.9890. Statistical parameters of the calibration curves may be found in Table 1. Standards containing greater than 1600 μg/L of acetol were also tested, and a tendency towards curvature was observed, indicating detector saturation. It is thus recommended that calibration curves and samples be diluted to within the linearity range stated above.

Accuracy was determined by preparing samples of propylene glycol spiked with acetol at approximately 3–12 mg/L, chosen to represent one to four equivalents of the estimated background level of acetol present in the lot of propylene glycol used. These samples were then diluted with water, prior to derivatization with PFBHA, to achieve assay spiking concentrations of approximately 250–1000 μg/L of acetol. Standards of acetol in

Table 1
Statistical parameters for the linearity regression curves

Parameter	UV	MS
$y = ax + b$	$141.029x + 3714.346$	$24676.91x - 111266.98$
r^2	0.9890	0.9944
Standard error of slope	4.703	584.38
Standard error of intercept	2845.761	353630.08

Table 2
Accuracy experiments showing recovery of acetol spiked into propylene glycol samples after derivatization with PFBHA

Acetol assay concentration (μg/L)	Spiked acetol concentration (μg/L)	Recovery via MS data (%)	R.S.D. via MS data (%)	Recovery via UV data (%)	R.S.D. via UV data (%)
190	0	N/A	2.1	N/A	3.2
440	250	100.5	8.8	125.3	3.7
690	500	91.9	16.9	99.7	5.4
1190	1000	85.0	2.3	93.9	0.6

water, at assay concentrations of approximately 250–1250 μg/L, were also prepared and derivatized. A calibration curve was constructed using integrated peak areas from MS and UV chromatograms of the acetol in water standards. Recovery for spiked samples of propylene glycol was calculated from the calibration curve, after correcting for background acetol in the matrix. Recovery data are shown in Table 2, and demonstrate a clear downward trend in recovery at higher acetol concentrations. This phenomenon was described previously and further demonstrates the advantage of targeting assay acetol concentrations to below 1000 μg/L.

Method precision was estimated based on the R.S.D. (%) of six replicate injections of a single 500 μg/L solution of derivatized acetol in acetonitrile–water (5:95, v/v), and was found to be 12.12% and 0.37% by MS and UV analysis, respectively. Method precision in the presence of excipient was also determined from the R.S.D. (%) of six replicate injections of a single derivatized solution of propylene glycol. Analysis by MS and UV gave R.S.D. (%) of 11.00% and 1.67%, respectively. These values compare favourably with other studies that have employed PFBHA for the derivatization of carbonyl compounds [1,3,6,9,12,16,20,22,25].

The limits of detection (LOD) and quantitation (LOQ) were calculated based on R.S.D. (%) of the integrated peak area for six replicate injections of a 55 μg/L solution of derivatized acetol using signal-to-noise ratio criteria of 3:1 for LOD and 10:1 for LOQ. LOD and LOQ were determined to be 12.5 and 37.5 μg/L, respectively. This LOD value was lower than those found in a similar study employing PFBHA derivatization and HPLC analysis of carbonyl compounds [9]. While lower values of LOD have been documented, the use of liquid–liquid [6] or liquid-phase microextraction [16] greatly complicated sample preparation as compared to the current method. Should lower detection limits be desired, pre-concentration via SPE (or some other technique) may be performed prior to analysis.

3.5. Determination of acetol in propylene glycol and glycerol from various suppliers

The acetol concentrations in seven samples of propylene glycol and one sample of glycerol were determined using this method, using both MS and UV data, and are shown in Table 3. Acetol assay concentrations were targeted to 200–700 μg/L. Glycerol was found to contain a much higher concentration of acetol than any of the propylene glycol samples, which themselves showed considerable variation based on manufacturer and lot. UV and MS estimates of acetol concentrations in samples of propylene glycol agreed fairly well, although the estimates diverged for the glycerol sample. This may result from glycerol contamination of the MS source, as discussed previously.

Table 3
Estimations of acetol concentration in commercial samples of propylene glycol and glycerol

Sample	Estimated acetol concentration in stock (mg/L)	
	Via MS data	Via UV data
Propylene glycol A	20.3	25.7
Propylene glycol B	16.3	18.7
Propylene glycol C	7.3	8.4
Propylene glycol D	3.1	2.8
Propylene glycol E	4.5	3.9
Propylene glycol F	4.0	5.1
Propylene glycol G	3.6	4.2
Glycerol A	132.3	99.9

3.6. Determination of the presence of other electrophilic impurities

Preliminary studies were conducted to determine the applicability of this method to the analysis of other electrophilic compounds which may also be present as excipient impurities. While quantitative analyses were not performed, PFBHA–carbonyl oximes of acetone, acetaldehyde, butanone, propionaldehyde, and benzaldehyde could be separated and identified by unique ion masses and comparison to standard chromatograms. Formaldehyde was also derivatized and detected in isolation. Chromatographic data are displayed in Fig. 3, while retention time and mass spectral data can be found in Table 4. In all cases, the PFBHA oxime was present as the

Table 4
Retention times and mass spectral data for PFBHA–carbonyl oximes

PFBHA oxime	Retention time (min)	[M+H] + (*m*/*z*)	Other MS ions (*m*/*z*)
Acetol	N/A	270	181, 222, 252
Formaldehyde	16.50	228	181, 197, 222, 269
Acetaldehyde	17.4, 17.7	240	181, 197, 222
Acetone	18.7	254	181, 222
Propionaldehyde	19.7	254	181, 197, 222, 305
Butanone	20.8, 20.4	268	181, 222
Benzaldehyde	23.0	302	181, 197, 333, 391

Where both E- and Z-isomers were detected, the retention time of the larger peak is listed first.

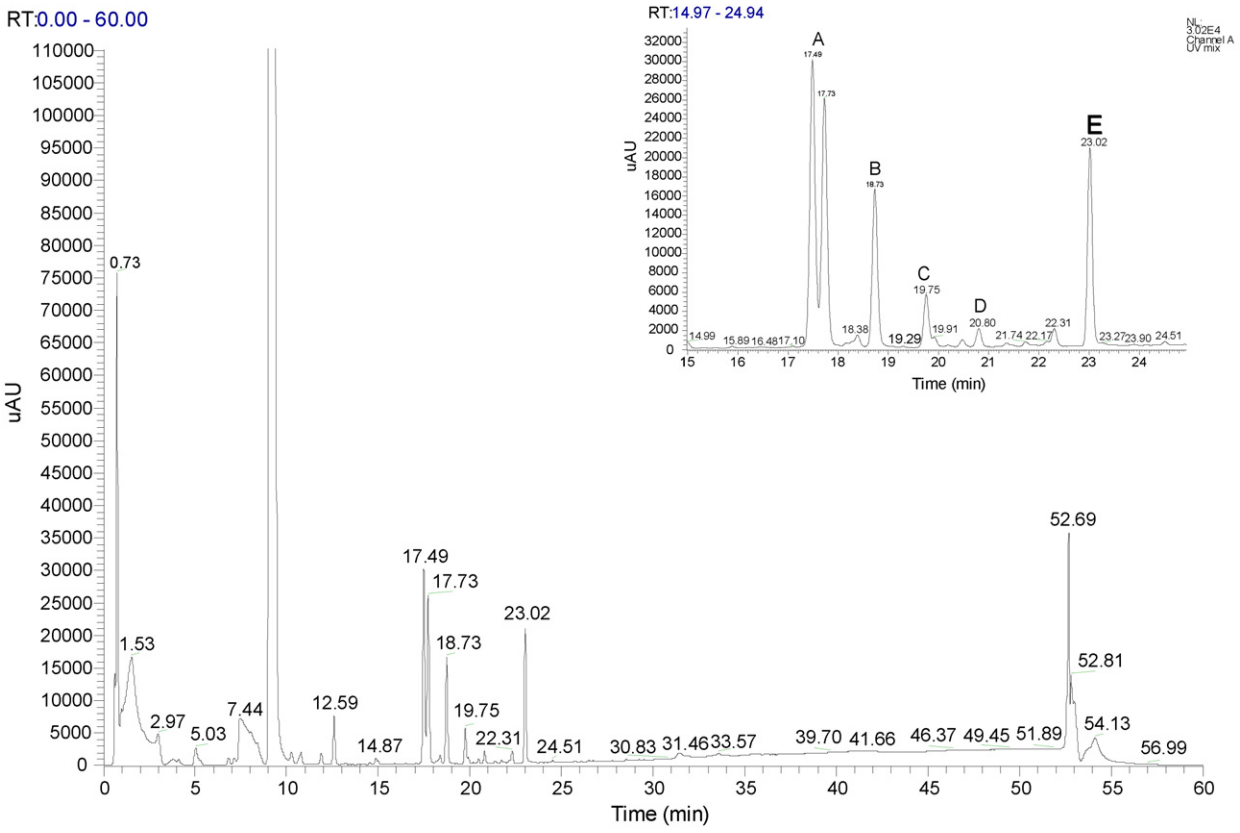

Fig. 3. Chromatogram of a mix of PFBHA–carbonyl oximes. The large peak with a retention time of approximately 9 min represents unreacted PFBHA. The insert shows a zoom into the area from 15 min to 25 min, with PFBHA–carbonyl peaks labelled (A = acetaldehyde, E- and Z-isomers; B = acetone; C = propionaldehyde; D = butanone, E- and Z-isomers; E = benzaldehyde).

$[M+H]^+$ ion. An ion of $m/z = 181$ was common to all spectra, likely representing a pentafluorobenzyl fragment.

These data highlight the potential expansion of this work to other applications. This method couples a simple sample preparation scheme that does not require sample concentration or post-derivatization extraction with a relatively fast room temperature derivatization scheme in aqueous solution, using inexpensive and widely available reagents. The quantitative nature of the method, along with the demonstrated ability to detect and identify a variety of electrophilic impurities, suggests a broader application to the development of an excipient screening assay. To the best of our knowledge, the current literature lacks such a versatile yet simple method for the determination of electrophilic impurities in pharmaceutical excipients.

4. Conclusions

The method presented herein provides a means for determining the concentration of acetol impurities in common pharmaceutical excipients. Sample preparation is simple to perform, without the need for pre-concentration or extraction prior to analysis, and derivatization occurs in aqueous solution at room temperature. Selectivity is based on the presence of a unique parent ion at $m/z = 270.0 \pm 0.5$ and an MS/MS fragment ion at $m/z = 252$, and can be further verified by UV detection at $\lambda = 228$ nm. LOD and LOQ were calculated as 12.5 μg/L and 37.5 μg/L, respectively, although further sensitivity could be achieved through sample concentration with SPE after PFBHA derivatization, if needed. This method was used to quantify the concentration of acetol impurities in samples of propylene glycol and glycerol, which was found to vary considerably between different suppliers and lots. A mixture of five other electrophilic compounds were also separated using this technique, suggesting that a more broad application for impurity screening in excipients may be possible.

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