

ORIGINAL ARTICLE

Selective determination of dimenhydrinate in presence of six of its related substances and potential impurities using a direct GC/MS method



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ABSTRACT

A novel simple, direct and selective gas chromatography–mass spectrometry (GC/MS) procedure was developed for the determination of the antihistamine drug dimenhydrinate (DMH) in presence of six of its related substances and potential impurities, namely, diphenylmethane, diphenylmethanol, benzophenone, orphenadrine, caffeine and 8-chlorocaffeine. The method involved resolution of the underivatized compounds using a trifluoropropylmethyl polysiloxane (Rtx-200) capillary column and the mass spectrometric detection was carried out in the electron-impact (EI) mode. Excellent baseline separation of DMH and the cited related substances was achieved in less than 15 min. Quantification of the parent drug DMH was based on measuring its peak area. The reliability and analytical performance of the proposed method were validated with respect to linearity, range, precision, accuracy, specificity, robustness, detection and quantification limits. Calibration curve of DMH was linear over the range 50–500 µg/mL with determination coefficient (R^2) = 0.9982. The proposed method was successfully applied for the assay of DMH in tablets dosage form with recoveries >96.80%.

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Introduction

Dimenhydrinate (DMH) is the diphenhydramine salt of 8-chlorotheophylline. It is chemically known as a (1:1) compound of 8-chloro-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione with 2-(diphenylmethoxy)-*N,N*-dimethylethanamine [1]. DMH is an antihistamine with antimuscarinic and significant sedative effects [2]. It is mainly used as an antiemetic in the prevention and treatment of motion sickness [2]. It is also used for the symptomatic treatment of nausea and vertigo caused by

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Ménière's disease and other vestibular disturbances [2]. The quantification of DMH in various drug formulations and/or biological samples was addressed in several reports. Analytical methodology in these reports involved the use of spectrophotometry [3,4], adsorptive stripping voltammetry [5], capillary electrophoresis [6], liquid chromatography–tandem mass spectrometry (LC/MS/MS) [7], high performance liquid chromatography (HPLC) with fluorescence detection [8], HPLC with UV detection [9] and high performance thin layer chromatography (HPTLC) [10,11]. Recently, an electrochemical method based on batch injection analysis with multiple pulsed amperometric detection was reported for stoichiometric determination of DMH [12].

Few GC/MS methods could be found in the scientific literature for the analysis of DMH merely for toxicological and forensic purposes [13,14]. Furthermore, few articles described the application of gas chromatography (GC) in analysis of diphenhydramine which is one of the two components of DMH. The simultaneous determination of diphenhydramine and pseudoephedrine in cough syrup was carried out using GC with flame ionization detector (FID) [15]. On the other hand, GC coupled with either nitrogen–phosphorus detection (NPD) or mass selective detection (MSD) was exploited for the determination of diphenhydramine in several biological samples [16,17]. Finally, GC/MS was applied for the identification of diphenhydramine and its metabolites along with other drugs commonly detected in clinical toxicology laboratories [18].

Although not widely used in pharmaceutical analysis, GC/MS can be considered one of the powerful techniques for separation and analysis of complex mixtures. We

previously published the selective determination of some pharmaceutical compounds in presence of their related substances using direct GC/MS methods [19,20]. Reviewing the literature revealed the use of gradient elution RP-HPLC with UV detection methods for the separation of DMH together with its related substances and impurities [21,22]. DMH related substances which are involved in this study are diphenylmethane, diphenylmethanol (benzhydrol), benzophenone, orphenadrine, caffeine and 8-chlorocaffeine [21–23]. Chemical structures of the parent drug and the related substances are shown in Fig. 1. To the best of our knowledge, there were no reports for the application of GC/MS in the analysis of DMH in pharmaceutical preparations. Additionally, there were no GC reports for the selective determination of DMH in presence of its related substances, degradation products or impurities. This work describes a simple, direct and selective capillary GC/MS method for the separation of DMH together with the above-mentioned related substances. The described method proved to be suitable for the quality assessment of DMH in its tablets.

Experimental

Materials

Dimenhydrinate, diphenylmethane, diphenylmethanol (benzhydrol), diphenylmethanone (benzophenone), orphenadrine hydrochloride, caffeine and 8-chlorocaffeine were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). HPLC-grade acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Pharmaceutical preparation examined in this study is Dramamine® tablets (Batch No. 133542, Prestige Brands, Inc., Tarrytown, NY, USA) labeled to contain 50 mg DMH per tablet.

General procedure and construction of calibration graph

DMH and its related substances are all readily soluble in acetonitrile. DMH (1000 µg/mL) stock solution was prepared in acetonitrile. DMH working solutions were prepared by dilution of aliquots of the stock solution with acetonitrile to reach the concentration range 50–500 µg/mL. The prepared working solutions were analyzed under the described GC/MS conditions under Instrumentation. The peak areas were plotted against the corresponding concentrations to construct the calibration graph.

Stock solutions of the six related substances (1000 µg/mL) were separately prepared in acetonitrile. Aliquots of these stock solutions were added to an aliquot of the parent compound stock solution and the mixture was diluted with acetonitrile. This mixture was analyzed under the described GC conditions.

Assay of tablets dosage form

A total of 10 tablets were weighed and finely powdered. Acetonitrile (30 mL) was added to an accurately weighed quantity of the powdered tablets equivalent to 50 mg DMH, stirred for 10 min then filtered into a 50-mL calibrated flask. The residue was washed with 2 × 5 mL acetonitrile and washings were

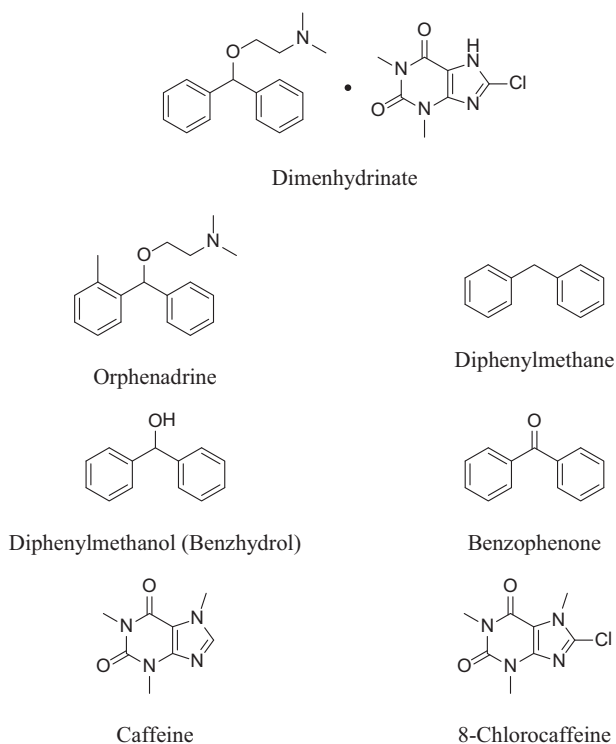


Fig. 1 Structures of the parent compound dimenhydrinate and the related substances involved in the study.

added to the filtrate and diluted to final volume with acetonitrile. Aliquots of the tablet solution were diluted with acetonitrile to obtain final concentrations within the range 50–500 µg/mL DMH and then treated as under general procedure.

Instrumentation

The GC/MS system consisted of an Agilent Technologies (Santa Clara, CA, USA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 5975C VL Agilent mass selective detector. The injection volume was 1 µL. The mass spectral scan rate was 2.86 scans per second. The GC was operated in splitless mode with a carrier gas (helium grade 5), flow rate was 0.7 ml/min and a column head pressure of 10 psi. The mass spectrometer was operated on the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230 °C. The GC injector was maintained at 300 °C and the transfer line at 275 °C. The temperature program used consisted of an initial temperature hold at 100 °C for 1 min, ramped up to 180 °C at a rate of 25 °C/min and holding the temperature at 180 °C for 8 min then the temperature was ramped up to 250 °C at a rate of 25 °C/min. The mass spectra were obtained by background subtraction and are the average of at least five scans. The chromatographic separation was carried out on a 30 m × 0.25 mm-i.d. column coated with 0.25 µm trifluoropropylmethyl polysiloxane (Rtx-200) purchased from Restek Corporation (Bellefonte, PA, USA). The study was carried in the GC/MS laboratory of Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University

Results and discussion

Development of the GC/MS method

The primary goal of this study was to provide a direct, fast and reliable method for the separation and quantification of DMH in presence of its related substances and impurities. In this regard, several non-polar capillary GC columns were evaluated in an effort to find the appropriate stationary phase providing the optimum separation. The investigated stationary phases included the 5% diphenyl/95% dimethyl polysiloxane (Rtx-5 Amine), 100% dimethyl polysiloxane (Rtx-1) and trifluoropropylmethyl polysiloxane (Rtx-200). The tested capillary GC columns were obtained from Restek Corporation (Bellefonte, PA, USA). Excellent separation of all the peaks within short analysis time was only obtained with the trifluoropropylmethyl polysiloxane (Rtx-200) column. Other columns failed to separate peaks of the structurally related compounds benzhydrol and benzophenone, in addition, insufficient resolution was observed between the peaks of DMH and 8-chloro-caffeine upon using the Rtx-1 column. Another positive feature of the Rtx-200 column was the relatively shorter retention times for all the peaks, e.g., retention time of the parent drug DMH on the Rtx-200 column was 35% and 25% shorter than those obtained with the Rtx-5 Amine and Rtx-1 columns respectively. Several temperature programs were evaluated and a program showing the best resolution in a reasonable analysis time was selected. The optimized temperature program started with an initial hold at 100 °C for 1 min, ramped up to 180 °C at a rate of 25 °C/min, held at 180 °C for 8 min then ramped to

250 °C at a rate of 25 °C/min. Programs with higher initial temperatures or higher ramp rates led to poor resolution especially between the two related substances (caffeine and 8-chloro-caffeine), while lower temperature ramps resulted in longer retention times and excessive peak tailing. It is noteworthy to mention that DMH produces 2 peaks at 7.60 and 14.01 min corresponding to its two components diphenhydramine and 8-chlorotheophylline respectively. Column performance can be expressed by the number of theoretical plates (*N*) which equals about 8150 and 9890 for diphenhydramine and 8-chlorotheophylline respectively. Tailing factors for these 2 peaks are 1.04 and 0.86 respectively. The studied related substances eluted at retention times 4.24, 5.82, 6.55, 8.51, 11.90 and 12.13 min for diphenylmethane, benzhydrol, benzophenone, orphenadrine, caffeine and 8-chloro-caffeine, respectively. Resolution was calculated between each of the two peaks of DMH and their nearest related substances' peaks. Resolution was found not less than 2.02, which represents successful and complete baseline separation. A typical GC chromatogram for the separation of DMH and its related substances is shown in Fig. 2. Mass spectrometry is a very useful technique for providing confirmation of chemical structure. The electron-impact (EI) mass spectrum can be considered a molecular fingerprint for the identification of compounds. Retention times as well as masses of the molecular ions and base peak fragments of the studied compounds are listed in Table 1.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines on validation of analytical procedures [24].

Linearity and concentration range

As mentioned earlier, DMH produces 2 well separated peaks (Fig. 2), however, the peak corresponding to diphenhydramine is much better in shape and provides higher area values and more reliable measurement than that of 8-chlorotheophylline; therefore quantification of DMH was based on measurement of diphenhydramine peak areas. The same principle was applied in previous publications dealing with the HPLC analysis of DMH based on the diphenhydramine component only [7,8]. The linearity of the proposed GC procedure was evaluated by analyzing a series of different concentrations of DMH (*n* = 8) over the concentration range 50–500 µg/mL. The following linear regression equation was generated by least squares treatment of the calibration data: $A = -942752 + 22958C$, ($r^2 = 0.9982$), where *A* and *C* are the peak area and concentration of DMH respectively. Other statistical parameters such as standard deviations of the intercept (*S_a*) and the slope (*S_b*) were calculated: 111,731 and 396 respectively. Consequently, deviation around the slope was evaluated by calculation of the RSD% of the slope (*S_b*%) which was found 1.73%. Finally, confidence intervals of the intercept and the slope were -1216,149 to -669,356 and 21,989–23,926 respectively.

Limits of detection and quantification

The limit of detection (LOD) is defined as the concentration that has a signal-to-noise ratio of 3:1, while for limit of

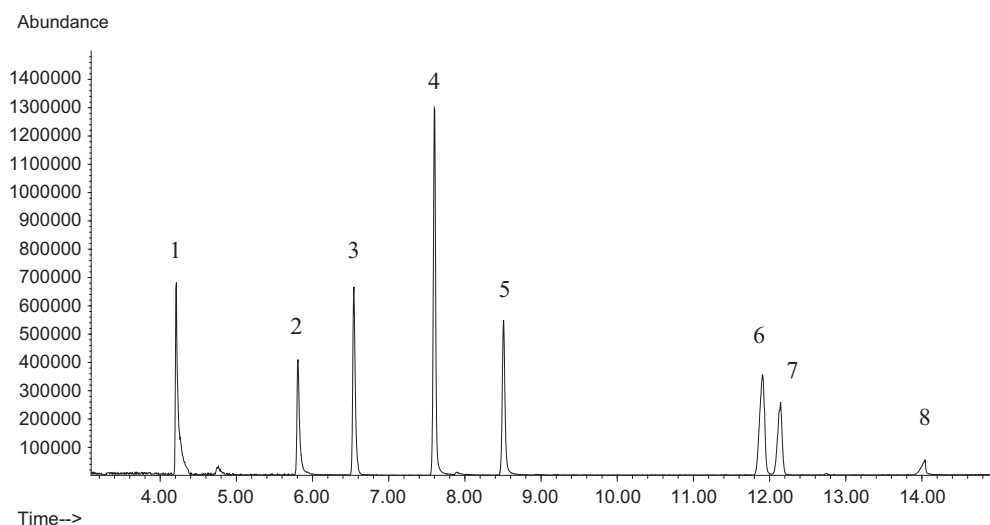


Fig. 2 GC/MS chromatogram of a mixture containing diphenylmethane (1), benzhydrol (2), benzophenone (3), DMH (diphenhydramine) (4), orphenadrine (5), caffeine (6), 8-chlorocaffeine (7) and DMH (8-chlorotheophylline) (8).

Table 1 Retention times and important mass fragments of the studied compounds.

Compound	Retention time (min)	Molecular ion (m/z)	Base peak (m/z)
Diphenylmethane	4.24	168	167
Benzhydrol	5.82	184	105
Benzophenone	6.55	182	105
DMH (diphenhydramine)	7.60	255	58
Orphenadrine	8.51	269	58
Caffeine	11.90	194	194
8-Chlorocaffeine	12.13	228	228
DMH (8-chlorotheophylline)	14.01	214	214

quantification (LOQ) the ratio considered is 10:1. The LOD and LOQ values of DMH were calculated and they were found to be 9.31 and 31.03 $\mu\text{g/mL}$ respectively which can be considered sufficient for quality control analysis of DMH in its pharmaceutical formulation.

Precision and accuracy

The within-day (intra-day) precision and accuracy for the proposed method were studied at three concentration levels for DMH using three replicate determinations for each concentration within one day. Similarly, the between-day (inter-day) precision and accuracy were tested by analyzing the same three

concentrations using three replicate determinations repeated on three days. Recoveries were calculated using the corresponding regression equation and they were satisfactory. The percentage relative standard deviation (RSD%) and percentage relative error ($E_r\%$) did not exceed 3% illustrating the acceptable precision and accuracy of the developed method for the estimation of DMH (Table 2).

Specificity

Specificity is defined as the ability to access unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and

Table 2 Precision and accuracy for the determination of DMH using the proposed GC/MS method.

	Nominal value ($\mu\text{g/mL}$)	Found \pm SD ^a ($\mu\text{g/mL}$)	RSD (%) ^b	E_r (%) ^c
Within-day	100	100.22 \pm 2.39	2.39	0.22
	200	197.12 \pm 3.42	1.74	-1.44
	400	389.67 \pm 6.95	1.78	-2.58
Between-day	100	98.88 \pm 2.78	2.81	-1.12
	200	196.25 \pm 4.04	2.06	-1.87
	400	396.11 \pm 7.47	1.89	-0.97

^a Mean \pm standard deviation for three determinations.

^b %Relative standard deviation.

^c %Relative error.

matrix components [24]. Method specificity was already demonstrated by the successful resolution of DMH from its related substances and potential impurities (Fig. 2).

Robustness

The robustness of an analytical procedure is a measure of its capability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage [24]. Robustness was examined by evaluating the influence of small variations in the injector temperature ($300 \pm 5^\circ\text{C}$), starting oven temperature ($100 \pm 5^\circ\text{C}$) and ramp of both steps in the applied temperature program ($25 \pm 2^\circ\text{C}/\text{min}$). These variations did not have any significant effect on retention times of all the separated peaks or the measured response (peak area of DMH). RSD% for retention times of any of the separated peaks and DMH peak area did not exceed 5% and 3% respectively. Additionally, these minor experimental changes did not affect the resolution between any of the separated compounds.

Stability of solutions

Acetonitrile was found to be the appropriate solvent for preparing both stock and working solutions of the parent drug and its related substances in this study. Stability of working solutions in acetonitrile was tested and they were found to be stable for at least 3 days at room temperature. Retention times of the studied compounds remained unchanged and no degradation was observed during this period. Additionally, the DMH percentage recoveries were not less than 96%.

Analysis of tablets dosage forms

The developed GC/MS method was applied for the assay of DMH in its commercial pharmaceutical formulation (Dramamine® tablets). DMH was extracted from the tablet powder using acetonitrile then dilution was done with the same solvent to reach the final concentration levels. No interfering peaks were observed from any of the excipients of the assayed tablets. A representative chromatogram obtained from the tablet extract is shown in Fig. 3. The direct determination of DMH in its tablets without any interference from the inactive

Table 3 Application of the proposed GC/MS method to the analysis of DMH in Dramamine tablets.

Parameters	GC/MS method	Reference HPLC method
%Recovery \pm SD ^a	98.85 \pm 1.84	98.46 \pm 1.17
RSD% ^b	1.86	1.19
<i>t</i>	0.40	
<i>F</i>	2.48	

Theoretical values for *t* and *F* at *P* = 0.05 are 2.31 and 6.39, respectively.

^a Mean \pm standard deviation for five determinations.

^b %Relative standard deviation.

ingredients can be considered further evidence for the simplicity and selectivity of the proposed method. Results obtained were precise, accurate and in good agreement with the label claim as displayed by the adequate values of percentage recoveries, standard deviation and RSD% (Table 3).

The USP reference liquid chromatographic method was adopted for the estimation of DMH in its tablets [25]. Recovery data obtained from the proposed GC/MS method were statistically compared with those of the reference USP method using the Student's *t*- and the variance ratio *F*-tests. In both tests, the calculated values did not exceed the theoretical ones at the 95% confidence level which indicated that there were no significant differences between the proposed and the reference method (Table 3). It can be concluded that the satisfactory analytical performance of the method fortifies its aptness for the routine and selective analysis of DMH in quality control units.

Conclusions

In this study, a simple, rapid and selective GC/MS procedure for determination of dimenhydrinate (DMH) in presence of six of its related substances was established. The method is considered direct using only acetonitrile as a solvent for preparation of both stock and working solutions. The samples did not require derivatization or pretreatment and the chromatograms showed excellent resolution between the parent compound and

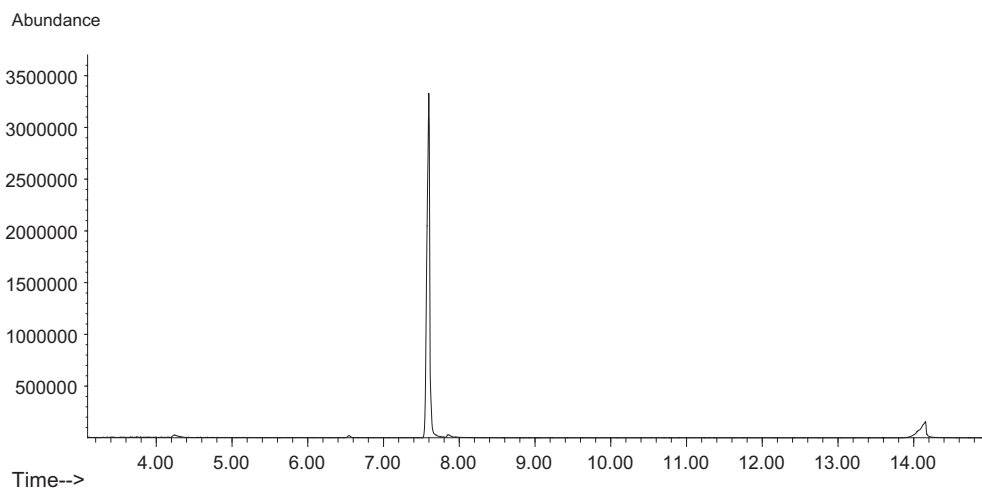


Fig. 3 GC/MS chromatogram of a solution containing 400 $\mu\text{g}/\text{mL}$ DMH obtained from Dramamine tablets.

its potential impurities. Very few GC/MS methods can be found in the scientific literature for the analysis of DMH merely for toxicological and forensic purposes. To the best of our knowledge, there are no GC/MS reports for determination of the drug in its tablets dosage form. Furthermore, very few stability indicating or impurity profiling methods for DMH could be found in the literature, and they depended only on HPLC. This is the first GC method developed for the selective determination of DMH in presence of its related substances and impurities. The method can be applied in two main fields: first; separation and identification of the expected impurities of DMH; second; the quality control assay of DMH tablet dosage form. In both applications, the method showed good analytical performance and acceptable accuracy and precision. Based on the results of this study, the GC/MS method can be used for testing DMH identity, assay, and purity.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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