Asian Journal of Chemistry; Vol. 23, No. 7 (2011), 2961-2964

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Multi-Residue Analysis of Four Volatile Organic Solvents in Omeprazole Materials Using Capillary Gas Chromatography

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(*Received*: 9 August 2010; *Accepted*: 9 March 2011) AJC-9707

A new multi-residue analytical method was developed for the simultaneous determination of four volatile organic solvent residues (acetonitrile, methanol, acetone and methylene chloride) in omeprazole materials by capillary gas chromatography with *n*-hexane as an internal standard. Direct injection and fast temperature-programmed mode were employed in this work and a PEG-20M flexible fused quartz capillary column (30 m × 0.25 mm × 0.33 μm) was used as the analytical column with FID detection. The influence of several important parameters on separation and detection of the four target analytes were carefully investigated. Under the optimum condition, good linearity were obtained in a range of 0.0007-0.9 mg/mL for all with the correlation coefficients (r^2) > 0.999. The average recoveries of all target analytes from omeprazole materials at three spiking levels were ranged from 94.5-107.9 % with the relative standard deviations less than 4.2 % (n = 5). The proposed method has the advantage of simplicity of operation, being fast and high recovery and is therefore an alternative tool to the existing methods for analyzing the solvent residuals in omeprazole products.

Key Words: Multi-residue analysis, Volatile organic solvents, Omeprazole materials, *n*-Hexane, Capillary gas chromatography.

INTRODUCTION

Residual solvents in synthetic drugs are the volatile organic compounds which are used or produced during the manufacturing process of drug substances or excipients. The residual solvents have no therapeutic effect on human disease but probably have toxic and carcinogenic effects on human body. So as far as possible, all of them should be removed to meet the demand for product specifications, GMP or other quality requirements[1,2]. But at present it is very difficult to remove it completely from the final products during the production process. In recent years, the toxicity and carcinogenesis of residual organic solvents in synthetic drugs are attracting increasing attention from many areas. Monitoring of residual solvents in pharmaceuticals is one of the hot topics and has become an important component of the drug quality control[3-5].

At present, most of the methods for determination of residual organic solvents are gas chromatography (GC)[6,7] and GC-MS[8] coupled with different sample pretreatment strategies, including headspace extraction[9-11], solid-phase microextraction[12,13] and ionic liquids extraction[14]. To increase the analytical methods convenience, a parallel dual-column system was used for preliminary identification and quantification of residual solvents in pharmaceuticals[15]. Recently, fast gas chromatography techniques were used for residual solvent analysis to shorten the analytical time[6,16]. However, simple and rapid multi-residue analytical methods for the rapid qualitative and quantitative determination of residual organic solvents in synthetic drugs are still desired strongly.

Omeprazole [6-methoxy-2-{(4-methoxy-3,5-dimethyl pyridin-2-yl)methylsulfinyl}-1*H*-benzo[d]imidazole] is a proton pump inhibitor to inhibits gastric acid secretion, which is widely used for the treatment of duodenal ulcer, gastric ulcer, reflux esophagitis and Zollinger-Ellison syndrome disease[17,18]. Several kinds of organic solvents including acetonitrile, methanol, acetone and methylene chloride are used in omeprazole's production process. Until now, there are few reports about multi-residue analysis of these volatile organic solvents in omeprazole materials and its products[19]. In this work, a simple and reliable multi-residue analytical method was developed for the simultaneous determination of the four volatile organic solvent residues in omeprazole materials by capillary gas chromatography with *n*-hexane as an internal standard. The proposed method is simple and sensitive and therefore as an alternative tool to the existing methods for analyzing the solvent residuals in omeprazole products.

EXPERIMENTAL

Omeprazole materials were obtained from Zhongnuo Pharmaceutical Co. Ltd. (Shijiazhuang, China). Acetonitrile, methylene chloride and methanol were purchased from Kermel Chemical Co. Ltd. (Tianjin, China). *n*-Hexane, methylene chloride, isopropanol, *n*-propanol, N,N-dimethyl formamide and dimethyl sulfoxide were purchased from Ding-Sheng Chemical Co. Ltd. (Tianjin, China). Acetone was obtained from Beifang-Tianyi reagent factory (Tianjin, China). All the other reagents used in the experiment were of the highest grade commercially available.

GC analysis: GC analysis was performed using a Shimadzu GC-2014 system equipped with a split/splitless injector and an FID detector (Shimadzu, Japan). GA-2000A air generator and SH-300 high-purity hydrogen generator were purchased from Zhongxing Huili Co. Ltd. (Beijing, China). A N-2000 Chromatography data workstation (Zheda Zhineng Co. Ltd., Hangzhou, China) was used as a data acquisition system. The analytical column was PEG-20M flexible fused silica capillary column (30 m × 0.25 mm × 0.33 μm) and its column flow rate was set at 1.5 mL/min with a split ratio of 10. High-purity nitrogen (99.999 %) was used as carrier gas with hydrogen as burn gas, air as aid-burn gas. The injection port and detection temperature was set to 230 and 250 ºC, respectively.

Preparation of sample and standard solution: The internal standard solution was prepared by dissolving 0.25 g *n*-hexane in 250 mL DMF. Stock standard solution of acetonitrile, methanol, acetone and methylene chloride was prepared individually by dissolving each standard compound in internal standard solution to get a concentration of 1.0 mg/mL. Aliquots of these stock standard solutions were combined to get the mixed stock solution and then diluted using internal standard solution by stepwise dilution method to get nine working solutions with different concentrations (0.9-0.0007 mg/mL).

Omeprazole material samples donated from the local pharmaceutical manufacturers were grinded into fine powder and then 0.06 g of each sample was dissolved and diluted to 5.0 mL with internal standard solution to get a final concentration of 12 mg/mL. The stability of the solutions was checked at 0, 12, 18 and 24 h after sample preparation in duplicate.

RESULTS AND DISCUSSION

Chromatographic parameters optimization: Main chromatographic parameters, including the input temperature, the columns with different polarity, oven temperature, the detector temperature and the split ratio were evaluated to get a desirable separation result of the target analytes. The input temperature must ensure that all samples can be vaporized to a fully vaporized state to avoid degradation. Generally, sample boiling point is a key factor to be considered when setting the input temperature. Compromising with the boiling points of solvent (DMF) and the target organic solvents, 230 ºC was employed in this work. The type and polarity of capillary chromatographic column also have different separation effect for the target analytes. When using KB-1 type column (100 % dimethyl polysiloxane as stationary phase) as analytical column, overlapping peaks were observed among acetonitrile, methanol, acetone and methylene chloride. However, on PEG-20M type column, the four target analytes could be separated completely with symmetry peaks (Fig. 1). Both column polarity and boiling point of the components were contributed to analytes retention behaviours on this column.

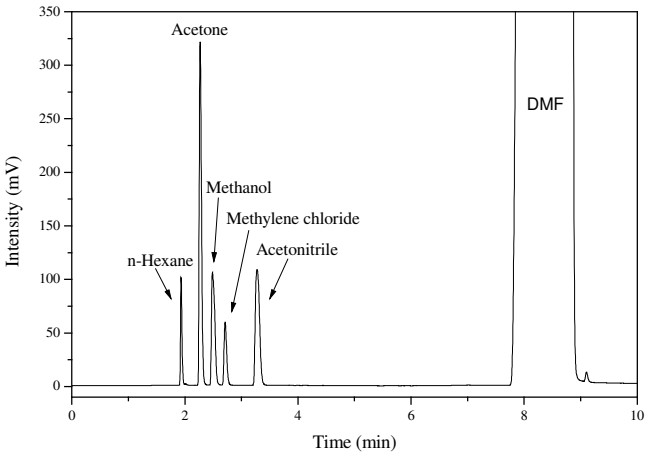

Fig. 1. Chromatogram of the four analytes solution on PEG-20M column

Direct injection mode and fast temperature-programmed techniques were applied in this work to shorten the analysis time. Although analyte elution rate increases linearly with flow rate, elution rate increases approximately exponentially with column temperature, thus, is far more effective in eluting strongly retained solutes. Considering the analytical time and resolution, a fast temperature programming profile was used in this work. The initial column temperature was set to 75 ºC for 1.0 min and then increasing at 5 ºC/min to 105 ºC and finally increasing at 15 ºC/min to 160 ºC for 5.0 min to wash out DMF and other interferences as much as possible.

Due to the fact that the sensitivity of FID detector is affected slightly by temperature, so the setting of the detector temperature was mainly considered keeping components vaporization state and 250 ºC was selected for further work. Additionally, the split ratios ranged from 1:10-1:30 were investigated experimentally. Compromising the sensitivity and separation efficiency, the split ratio of 1:15 was employed in this work.

Selection of sample solvent: A common strategy is proposed that the solvent of different polarity and surface energy affect the retention behaviours of the analytes. When water was employed as sample solvent, peak broadening was observed and the retention time of the analytes were shorten with the increasing of the duplicated times of injection, which was due to water have strong inter-action with the stationary phase of PEG-20M column. Better separation efficiencies were obtained when using DMF and DMSO as solvent, which was due to DMF and DMSO have good ability to dissolve and extract the four analytes. Furthermore, due to its high boiling point (153 ºC for DMF; 189 ºC for DMSO) and long retention time in column, it will enable the volatile components was flowing out firstly to avoid interferences. Considering the boiling point of the target analytes (81.6, 64.5, 56.5 and 39.8 ºC for acetonitrile, methanol, acetone and methylene chloride), DMF is enough to keep them test-first-out without interferences.

TABLE-2
CONTENTS OF SOLVENT RESIDUES IN OMEPRAZOLE MATERIALS (n = 5)

Acetonitrile (mg/g)	RSD (%)	Methanol (mg/g)	RSD (%)	Acetone (mg/g)	RSD (%)	Methylene chloride (mg/g)	RSD (%)
ND	–	0.176	1.9	0.00628	4.0	0.0123	4.3
ND	–	0.167	0.7	0.00499	3.2	0.0098	4.3
ND	–	0.149	0.4	0.00526	2.3	0.0133	3.0

Selection of internal standard substances: Seven kinds of internal standard substances (*n*-propanol, isopropanol, ethyl acetate, *n*-hexane, cyclohexanol, tetrahydrofuran and chloroform) were experimentally investigated. Under the optimized chromatographic condition, the peak of methanol, isopropanol and methylene chloride could not be separated completely. The similar phenomenon was observed for *n*-propanol and acetonitrile, ethyl acetate and methanol, tetrahydrofuran and methanol. The peak of chloroform was overlapped with DMF and the peak of cyclohexanol was partly overlapped with the impurities of DMF which have longer retention time (9.2 min) on column. The best internal standard substance is *n*-hexane, which have suitable retention time and baseline separation with the target analytes.

Validation of the methodology: Calibration curves were constructed using the rate of peak areas of analytes with the internal standard measured at nine increasing concentration, in a range of 0.0007-0.9 mg/mL. Good linearity was obtained for all analytes throughout the selected concentration range and the regression equations were shown in Table-1. The regression coefficients of the four linearity curves were > 0.999. Intra-assay and inter-assay precision expressed as the relative standard deviation (RSD) of concentrations calculated from the quality control samples on same day (n = 5) and five different days were less than 2.7 and 4.1 %. Based on a signal-to-noise ratio of 3, the limits of detection (LOD) for the four analytes ranged from 0.06-0.23 mg/L, respectively.

TABLE-1
LINEARITY EQUATION AND LOD OF THE FOUR ANALYTES

Analytes	Linearity equation	r^2	LOD (S/N = 3) (mg/L)	RSD (%)
Acetonitrile	Y = 3.249X – 0.0080	0.9997	0.12	2.8
Methanol	Y = 2.208X – 0.0074	0.9993	0.15	2.5
Acetone	Y = 4.107X + 0.0140	0.9995	0.06	1.9
Methylene chloride	Y = 0.9919X – 0.0012	0.9998	0.23	1.2

Sample analysis: Omeprazole materials were grinded into fine powder and 0.06 g was dissolved into 5.0 mL volumetric flask using internal standard solution for GC analysis. A typical chromatogram of omeprazole materials is shown in Fig. 2. No interfering peaks from the drugs matrix were observed at the retention time of the compounds of interest, which demonstrates the good selectivity of the developed separation strategy. The experimental results (Table-2) indicated that the content of the four volatile organic solvents determined in omeprazole materials was lower than that marked by the manufacturer. The stability of sample solutions was evaluated by the percentage of the relative peak area variations of acetonitrile, methanol, acetone and methylene chloride from time 0 h up to 24 h were between 97.3 and 103.6 %. Recovery was tested to investigate the effect of the actual sample matrix by spiking three different levels of the analytes into actual sample and the results were shown in Table-3. The means recoveries for all analytes were in a range of 94.5-107.9 % with SRD less than 4.2 %, which indicated the method was reliable and it was possible to quickly measure the multi-residue of the analytes in omeprazole products.

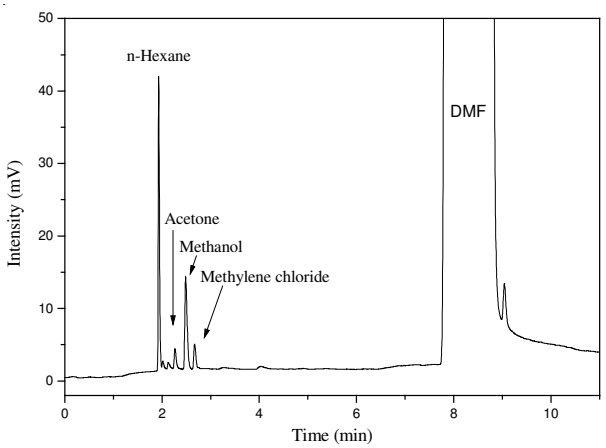

Fig. 2. Chromatogram of volatile organic solvents in omeprazole materials

TABLE-3
RECOVERIES OF THE FOUR ANALYTES IN OMEPRAZOLE MATERIALS (n = 5)

Analytes	C_{sample} (mg/g)	C_{adding} (mg/g)	$C_{measure}$ (mg/g)	Recovery (%)	RSD (%)
Acetonitrile	–	0.094	0.097	103.2	2.6
Methanol	0.176	0.105	0.275	94.5	4.2
Acetone	0.006	0.118	0.129	104.2	3.1
Methylene chloride	0.012	0.105	0.116	98.5	4.2
Acetonitrile	–	0.188	0.203	107.9	1.2
Methanol	0.167	0.210	0.367	95.2	3.6
Acetone	0.005	0.236	0.243	100.8	1.1
Methylene chloride	0.010	0.210	0.221	100.5	1.3
Acetonitrile	–	0.150	0.158	105.3	2.5
Methanol	0.149	0.168	0.314	98.2	3.7
Acetone	0.005	0.189	0.195	100.5	2.3
Methylene chloride	0.013	0.168	0.179	98.8	2.4

ACKNOWLEDGEMENTS

This research was supported by an Inha University Research Grant (INHA-2010).

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