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Method Development and Validation for the GC-FID Assay of Ethanol in Reservoir-type Fentanyl Transdermal Patches

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ABSTRACT

With ethanol acting as a skin permeation enhancer in the reservoir-type fentanyl transdermal delivery system, accurate assay of ethanol in the adapted cosolvent system is an important quality control component. This paper describes the development and validation of an isothermal gas chromatography-flame ionization detection (GC-FID) method for the assay of ethanol in reservoir-type fentanyl patches (Durogesic®). Samples were extracted with water by ultrasonic vibration using acetone as the internal standard. Using a polar column (Supelco OVI-G43, 30 m × 0.53 mm) for the gas chromatographic analysis, ethanol and excipients were well resolved. The method was fully validated according to the ICH Q2A and Q2B guidelines. The range of linearity for ethanol was demonstrated from approximately 0.1 to 0.9 mg/mL ($r^2 > 0.99$). The accuracy (recovery tests at 60, 100, and 140% of the nominal analytical concentration of 0.5 mg/mL) was determined in the range of 98~101% with RSD ≤ 0.75%. The precision (repeatability or intermediate precision) was calculated as RSD ≤ 1.21%. The detection and quantitation limits were determined to be 1.99 and 6.03 μg/mL, respectively. Furthermore, the robustness and system suitability testing were also considered. In conclusion, a validated method for the assay of ethanol in reservoir-type fentanyl transdermal patches was successfully applied to quality control practices.

Key words: transdermal patch, ethanol, GC-FID, method validation, quality control

INTRODUCTION

Fentanyl, a potent synthetic opioid, is a selective and pure agonist for the mu-opioid receptor and shares the typical opioid actions of morphine. The low molecular weight, high potency and lipid solubility of fentanyl make it be used suitably and increasingly in transdermal drug delivery systems. Due to special advantages of noninvasive parenteral administration, stable serum concentrations, high compliance and a potential lower rate of gastrointestinal adverse effects, fentanyl transdermal patches might be useful in patients with chronic pain of nonmalignant origin if strong opioids are indicated[1-3].

In Taiwan, Durogesic® reservoir patches (Figure 1) are the only marketed fentanyl patches at present. Each patch consists of four functional layers: (1) a backing layer of polyester film, (2) a drug reservoir of fentanyl and alcohol gelled with hydroxyethyl cellulose, (3) an ethylene-vinyl acetate copolymer membrane that controls the rate of fentanyl delivery to the skin surface, and (4) a fentanyl containing silicone adhesive[4,5]. In practice the ethanol/water cosolvent system was applicably used as the donor vehicle, and ethanol also acts as a skin permeation enhancer (flux enhancer) to deliver fentanyl in the transdermal patches[6]. In addition, loss of ethanol can be nearly eliminated with the form-fill-seal design in fentanyl transdermal systems. However, steady-state flux and cumulative amounts of fentanyl permeated through human epidermis would increase proportionally with the concentration of ethanol, from 0 up to 30% (w/w), in the donor solutions[7]. Even though both the United States Pharmacopeia (USP, 30th revision) and the European Pharmacopoeia (5th edition) do not have an analytical protocol for quality control of reservoir-type fentanyl patches[8,9], the assay of ethanol should be of essential in quality control to ensure the safe and efficient drug administration of the patches.

Gas chromatography (GC) is a powerful and widely used tool for the separation, identification and quantification of alcohols in various samples[10]. Nevertheless, packed glass columns, as mentioned in the USP, is not commonly employed in modern GC equipment. Most of the publications on the determination of ethanol content

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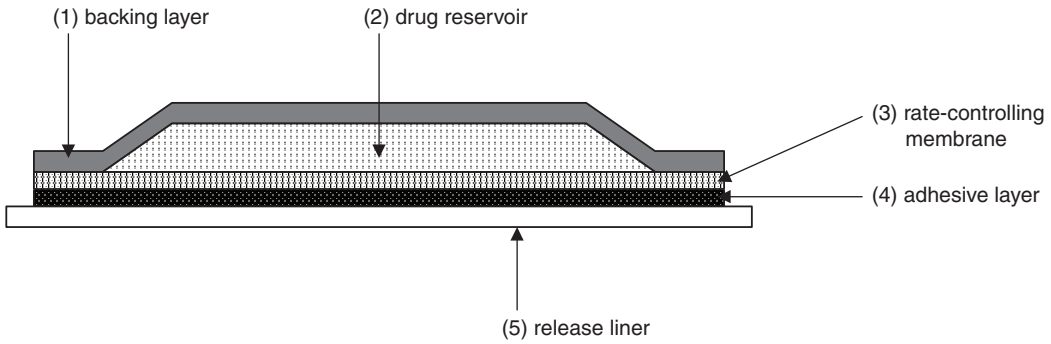

Figure 1. Schematic representation of functional layers in a Durogesic® reservoir type patch. The release liner of polyester film must be removed before using.

have been developed for liquid herbal drug preparations[11,12] or biological fluids[13-18] using headspace sampling technique coupled with GC–mass spectrometry or GC–flame ionization detection (GC-FID). However, the quantitative assays found in the literature are not appropriate for pharmaceutical samples, and thus conventional GC-FID was employed as the preferred choice from cost standpoints. Therefore, this study is aimed to develop a capillary GC-FID method for the determination of ethanol content in reservoir-type fentanyl patches.

MATERIALS AND METHODS

I. *Materials*

Solvents used were of ≥ 99.5% purity and purchased from the following sources: ethanol absolute (LiChrosolv, 99.9%), acetone (Uvasol, 99.9%) from Merck (Darmstadt, Germany); methanol (HPLC grade), acetonitrile (HPLC grade), 1,4-dioxane from Mallinckrodt (NJ, USA); 2-propanol, dichloromethane from Fluka (Seelze, Germany); chloroform from Riedel-deHaën (Seelze, Germany); trichloroethylene from Acros (Geel, Belgium); cyclohexane from Janssen (Geel, Belgium), benzene from Janssen (Geel, Belgium). Deionized water was prepared from Milli-Q system (Millipore, MA, USA). Reservoir-type fentanyl patches (Durogesic®) were from Janssen Pharmaceutica N.V. (Beerse, Belgium).

II. *Equipment*

The GC system consisted of a Model 6890N Series gas chromatographer equipped with an autosampler from Agilent Technologies (Palo Alto, CA, USA). The detection was performed by means of FID. Separation was achieved using a Supelco OVI-G43 (6% cyanopropylphenyl, 94% dimethylsilioxane) capillary column with the following dimensions, 30 m × 0.53 mm and 3 μm film thickness. The data was acquired via Chemstation Plus Software, Version A.08.03. A Mettler AT261 analytical balance was used for massing standards, and an Elma D-78224 sonicator was used for sample sonication. The chromatographic conditions were as follows: column, Supelco OVI-G43 (30 m × 0.53 mm, 3 μm); carrier gas, helium; detector, FID; injector temperature, 200°C; detector temperature, 250°C; oven temperature, 45°C (rate is 0°C/min, isothermal); flow rate, 5.3 mL/min; injection volume, 1.0 μL; split ratio, 20.0/1.0; quantitation, peak area; approximate retention time of ethanol, 3.2 min.

III. *Preparation of Solutions*

(I) *Solutions Used for Method Development*

Solutions of each solvent were prepared independently by dissolving each compound in ethyl acetate in order to obtain a final concentration of 1.0 mg/mL. A solution containing all solvents and internal standard, acetone, was also prepared in ethyl acetate to achieve 1.0 mg/mL for each compound to demonstrate the specificity of the method.

(II) *Standard Solutions for Method Validation*

Standard solutions were prepared as follows:

1. The internal standard solution contained 500 mg acetone in 100 mL water.

2. The stock standard solution contained 500 mg absolute ethanol in 100 mL water.

(III) *Sample Preparation*

Sample solutions were prepared as follows:

1. The extract media contained 125 mg acetone in 100 mL water.

2. The sample extraction was prepared by these steps:

The release liner was removed from one fentanyl patch and folded in half such that the adhesive side of the system is doubled over onto itself. The patch was transferred into a 100 mL serum bottle containing 50 mL of extract media. The patch was snipped using a small and sharp scissors into four equal parts below the surface of the extract media in the serum bottle. The bottle was sealed air-tight with a silicon rubber seal and a cap, and

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transferred to a water bath. The ethanol was extracted at about 50°C by ultrasonic vibrating for two hours.

3. The sample solution was prepared by diluting 20 mL of the resulting sample extraction to 50 mL with water.

Examples of standard and sample chromatograms were illustrated in Figure 2.

IV. *Validation*

The method was validated according to the ICH Q2A and Q2B guidelines on the validation of analytical methods[19,20]. The validation criteria such as specificity, linearity, range, accuracy, precision (repeatability and intermediate precision), detection limit, quantitation limit, robustness and system suitability testing were evaluated. Excel 2000 (Microsoft Office) was used for the statistical analysis.

RESULTS AND DISCUSSION

I. *Method Development*

Ethanol, C_2H_5OH, is a polar molecule, and therefore a polar column, OVI-G43, was used for the separation. The GC parameters used in the method development were based on the boiling point 78.5°C of ethanol. The injection port, detector and oven temperatures were set to 200, 250 and 45°C, respectively. The oven program is isothermal with the run time of 7 min. The head pressure was set to ensure a helium flow of 5.3 mL/min. The split was then adjusted to 20.0/1.0. The solvent, column and acquisition parameters were chosen to be a starting point for the method development. However, the chromatography produced using these starting parameters turned out to be excellent. The retention time of ethanol was approximately 3.2 min with good peak shape and USP tailing was approximately 1.1. So that no further optimization of the method was performed.

The preliminary precision and linearity studies performed during the method development showed that 1 μL injection volume using acetone as an internal standard was reproducible and the peak response was significant at the analytical concentration. The preliminary extraction study showed that recoveries did not significantly increase (< 5%) with more extraction media (100 mL) or longer extraction period (for 4 h or 24 h). In addition, it also indicated that sealing the bottle air-tight with a silicon rubber seal and a cap during extraction period is important to prevent experimental errors. Chromato-

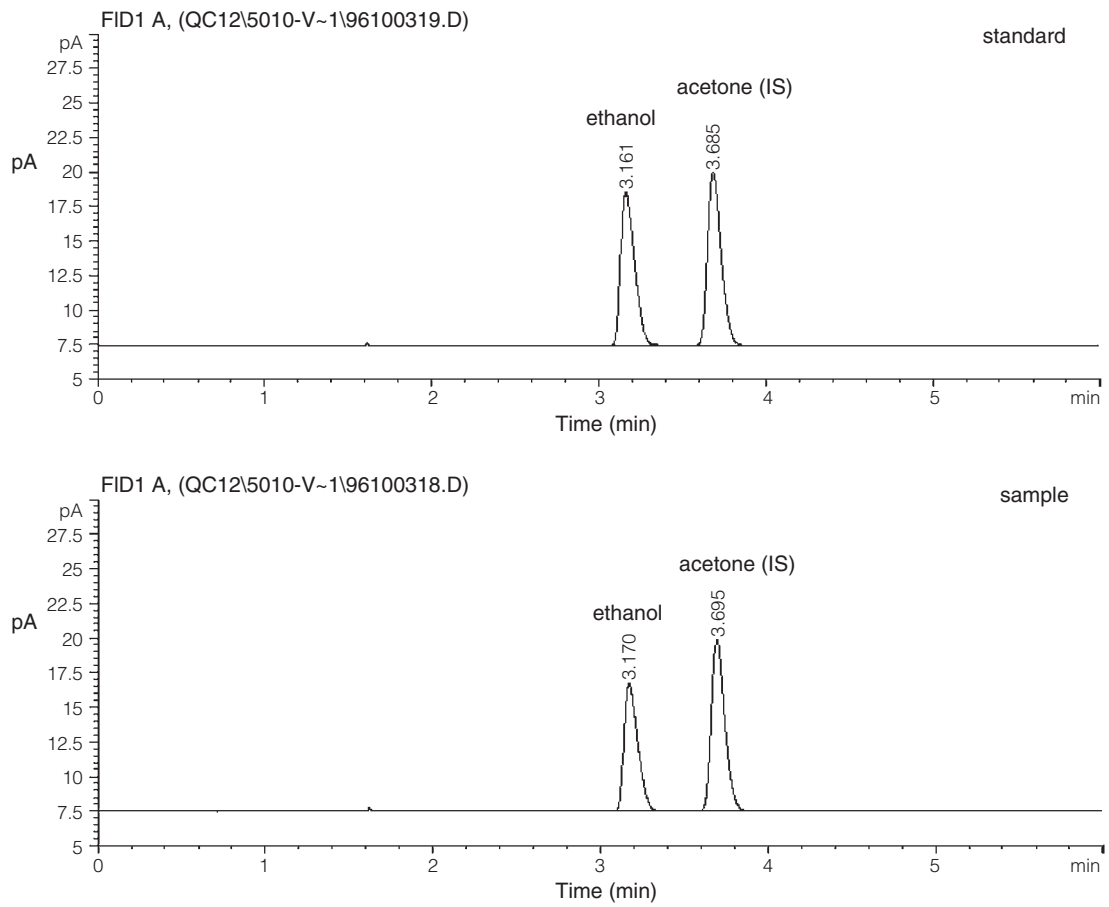

Figure 2. GC-FID chromatogram on capillary column by injecting 1.0 μL standard solution or sample preparation.

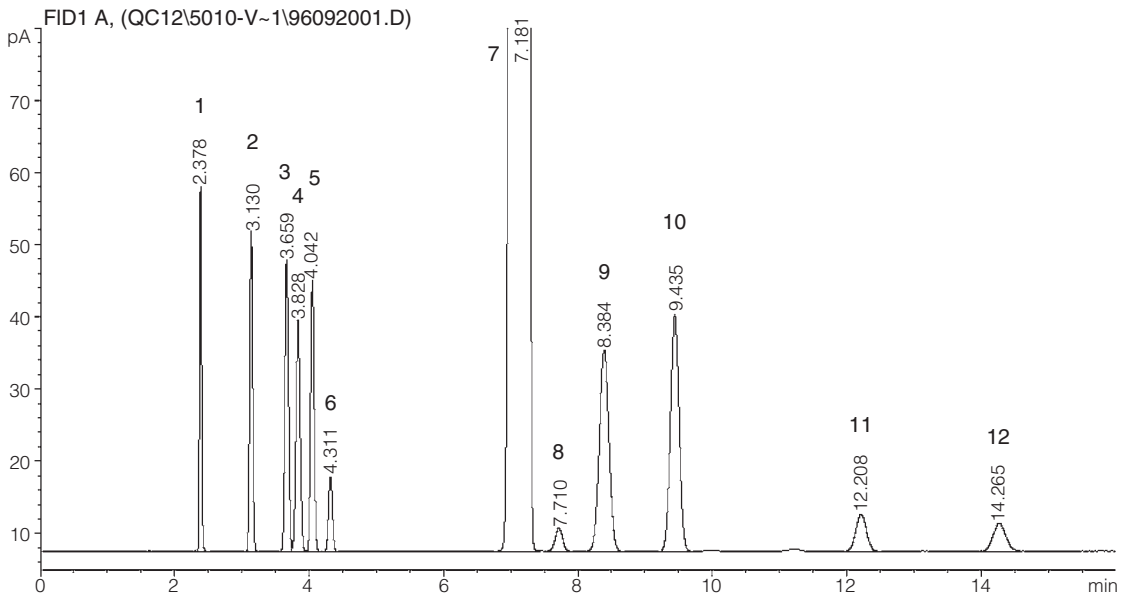

Figure 3. Chromatographic separation of all compound: (1) methanol; (2) ethanol; (3) acetone; (4) 2-propanol; (5) acetonitrile; (6) dichloromethane; (7) ethyl acetate; (8) chloroform; (9) cyclohexane; (10) benzene; (11) trichloroethylene; (12) 1,4-dioxane.

grams of the resulting solutions exhibited very good peak shapes for ethanol (USP tailing ca. 1.1) and co-elution of excipients was not observed.

Furthermore, the stability of ethanol and acetone in standard and sample preparation was evaluated by storing the solutions for 24 h, at room temperature (25 ± 5°C), and testing in triplicate at beginning and the end of the storage period. The results obtained were comprised between 98 and 102% of the initial value. No significant degradation of ethanol and internal standard was observed.

II. *Method Validation*

(I) *Specificity*

All twelve solvent components used for method development are well separated. The specificity of the method was clearly demonstrated in Figure 3 that illustrates the complete separation of the main solvents considered in this work and their corresponding contaminants.

(II) *Range of Linearity*

The linearity of peak area ratio versus concentration for ethanol was studied from approximately 0.1 up to 0.9 mg/mL. Five solutions were prepared corresponding to 20, 60, 100, 140 and 180% of the nominal analytical concentration of 0.5 mg/mL using the stock standard solution, and each of them contained acetone at 0.5 mg/mL as internal standard. At each level samples were injected and analyzed according to the method previously described by three analysts. For assessing the linearity, the regression equation and the determination coefficients (r^2) were calculated. The method was found to be suitable for a single point calibration, and the linearity parameters for ethanol were summarized in Table 1.

Table 1. Linearity parameters for ethanol

Analyst	Slope	Intercept	r^2
1	1.8387	0.0022	1.0000
2	1.8863	−0.0064	0.9988
3	1.8439	−0.0017	1.0000

(III) *Accuracy*

Accuracy refers to the percent of analyte recovered by an assay from a known added amount, hence the recovery of ethanol from the fentanyl patch was studied by assaying the sample preparation spiked with ethanol, corresponding to a final concentration of 60, 100, and 140% of the nominal analytical concentration of 0.5 mg/mL. At each level samples were injected in triplicate by three analysts, and the results are summarized in Table 2.

(IV) *Precision (Repeatability and Intermediate Precision)*

The repeatability and the inter-day intermediate precision were determined according to the above described accuracy test by three analysts on different days. All the twenty-seven determinations were obtained by injecting each sample in triplicate, and the RSD were reported also in Table 2.

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(V) *Detection and Quantitation Limits*

The detection and quantitation limits of ethanol in the present study were based on the standard deviation of the response and the slope, and estimated using the mean intercept of the calibration mode. Three solutions prepared at 50, 75 and 100 μg/mL ethanol in water were injected in triplicate. The detection limit and the quantitation limit were expressed as DL = 3.3σ/S and QL = 10σ/S (where σ = the standard deviation of y-intercepts of regression lines, S = the slope of the calibration curve). The result of DL was 1.99 μg/mL and QL was 6.03 μg/mL.

(VI) *System Suitability Testing*

As system suitability testing is an integral part of

Table 2. Recovery of ethanol from the fentanyl patch

			Recovery						Repeatability		Intermediate	
Analyst	Level (mg/mL)	Injection	Concn. Standard[a] (mg/mL)	Concn. Sample[b] (mg/mL)	Concn. Spike[c] (mg/mL)	Recovery (%)	Average $n = 3$	RSD (%)	Average $n = 9$	RSD (%)	Average $n = 27$	RSD (%)
1	0.3	1	0.3002	0.2489	0.2762	101.14	100.69	0.45	100.96	0.52		
		2	0.2992	0.2502	0.2750	100.23						
		3	0.3004	0.2495	0.2760	100.70						
	0.5	1	0.4983	0.4170	0.4600	100.95	101.27	0.75				
		2	0.4981	0.4170	0.4593	100.73						
		3	0.4978	0.4162	0.4623	102.13						
	0.7	1	0.6992	0.5872	0.6455	100.66	100.91	0.27				
		2	0.6998	0.5864	0.6462	100.88						
		3	0.7003	0.5872	0.6479	101.19						
2	0.3	1	0.3135	0.2513	0.2799	98.42	98.22	0.32	98.73	0.89	100.19	1.21
		2	0.3127	0.2517	0.2788	97.86						
		3	0.3129	0.2520	0.2799	98.39						
	0.5	1	0.5209	0.4213	0.4665	98.23	98.09	0.17				
		2	0.5190	0.4231	0.4663	98.18						
		3	0.5190	0.4225	0.4653	97.90						
	0.7	1	0.7253	0.5971	0.6606	99.83	99.87	0.15				
		2	0.7254	0.5972	0.6604	99.75						
		3	0.7247	0.5960	0.6605	100.04						
3	0.3	1	0.2974	0.2480	0.2749	101.47	101.17	0.39	100.89	0.32		
		2	0.2970	0.2479	0.2744	101.31						
		3	0.2972	0.2489	0.2741	100.72						
	0.5	1	0.4940	0.4140	0.4561	100.85	100.70	0.25				
		2	0.4938	0.4144	0.4562	100.83						
		3	0.4930	0.4156	0.4553	100.41						
	0.7	1	0.6921	0.5833	0.6403	100.74	100.81	0.11				
		2	0.6930	0.5827	0.6405	100.75						
		3	0.6916	0.5845	0.6413	100.94						

[a]Concn. Standard: determination of ethanol content by injecting each standard solution.
[b]Concn. Sample: determination of ethanol content by injecting each sample preparation.
[c]Concn. Spike: determination of ethanol content by injecting each spiked solution.

method development, these suggested limits were used as a reference to set up the initial system suitability criteria, including injection precision (RSD < 2.0% for n = 6), number of theoretical plate (N > 10000), tailing factor (T < 1.5), resolution (R > 1.5) and relative retention (α > 1.05). The data were summarized in Table 3.

(VII) *Robustness*

To show the reliability of an analysis with respect to deliberate variations in analytical parameters, the solutions used for method development were injected with changing the chromatographic condition setting of oven temperature at 44 to 46°C or flow rate at 5.0 to 5.6 mL/min, respectively. The consequence of the evaluation was good and ensured that the validity of the analytical procedure is maintained whenever used. The data were summarized in Table 4.

CONCLUSIONS

According to the ICH guidelines, the validation criteria such as specificity, range of linearity, accuracy, precision (repeatability and intermediate precision), detection limit, quantitation limit, system suitability and robustness were considered. Validation testing shows that the method is specific and linear in the range of 0.1 to 0.9 mg/mL. The accuracy and precision testing demonstrated a high degree of reproducibility confirmed by three analysts. Furthermore, the system suitability testing shows the reliability during normal usage; and the robustness study shows the method performance remained unchanged by small variations in analytical parameters. Consequently, the results presented in this study indicate the validated gas chromatographic method can be applied to the ethanol assay for the quality control of reservoir-type fentanyl patch.

Table 3. System suitability study

	Criteria	Results
RSD	< 2%	0.16%
N	> 10000	24035
T	< 1.5	1.06
R	> 1.5	11.33
α	> 1.05	1.70

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Table 4. Robustness study

	Oven temperature (°C)			Flow rate (mL/min)		
	44	45	46	5.0	5.3	5.6
N	23378	24035	24148	24506	24035	23139
T	1.06	1.06	1.06	1.06	1.06	1.06
R	11.41	11.33	11.07	11.39	11.33	11.11
α	1.70	1.70	1.68	1.69	1.70	1.70

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