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Journal of Chromatography B, 846 (2007) 264–272
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Validated GC–MS analysis for the determination of residual fentanyl in applied Durogesic® reservoir and Durogesic® D-Trans® matrix transdermal fentanyl patches

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Received 21 December 2005; accepted 6 September 2006
Available online 9 October 2006

Abstract

The method development and validation characteristics are described of a simple gas chromatographic–mass spectrometric (GC–MS) analytical procedure to determine residual fentanyl in used Durogesic® reservoir patches and Durogesic® D-Trans® matrix technology based systems to estimate the actual rate of transdermal fentanyl delivered in individual patients. The sample preparation protocol constituting a saline based extraction of sets of new patches of each nominal dose available, resulted in fentanyl extraction recoveries to increase steadily as a function of increasing extraction time. For the reservoir type transdermal therapeutic system (TTS), fentanyl extraction efficiencies at equilibrium (16 h) ranged from approximately 60% (100-μg/h TTS) to 95% (25-μg/h TTS), whereas for the matrix type system considerable lower recoveries were demonstrated for the highest nominal dose rates (35%–52%), while reaching 90% for the 25-μg/h system. For the latter type of fentanyl TTS, an optimized methanol based extraction protocol yielded virtually quantitative fentanyl recoveries for each matrix patch nominal dose level at substantially shorter extraction periods (15 min). The GC–MS analytical method using selected ion monitoring (SIM) and deuterated fentanyl as internal standard was shown to be adequately selective with regard to the presence of other compounds in the Durogesic® patches. It was further demonstrated that the developed analytical protocols provided highly reproducible and accurate estimates of the initial fentanyl content of each patch type at all available nominal doses, with coefficients of variation and relative errors generally below 10%. These advantageous assay validation characteristics can be further transposed to the application of residual fentanyl level estimates in used patches, provided that with each batch of samples also a set of new TTSs with equal dose is assayed to perfectly mimic extraction phenomena. Finally, the presented GC–MS analytical protocol was successfully applied for the determination of residual fentanyl in a subset of 57 reservoir type patches obtained from four palliative patients.

Keywords: Fentanyl; Transdermal patch; Reservoir type; Matrix type; Method validation; GC–MS

1. Introduction

Fentanyl is a potent synthetic opioid that is increasingly being used in transdermal drug delivery systems [1]. The transdermal therapeutic system (TTS) for fentanyl incorporating reservoir technology became available in most European countries in the early 1990s and was marketed under the name Durogesic®. The Durogesic® reservoir patch (Fig. 1A) consists of four functional layers: (1) a backing layer of polyester film, (2) a drug reservoir of fentanyl and alcohol USP 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 [2,3]. Each system is labelled with a nominal flux, which represents the average amount of drug delivered to the systemic circulation per hour across average skin [4]. The nominal flux is dependent upon the surface area of the patches, being available in four sizes, designed to release fentanyl at rates of 25, 50, 75 and 100 μg/h.

From the beginning of 2005, Durogesic® D-Trans®, a new matrix technology based fentanyl TTS, has become available in 16 European countries [5]. The fentanyl matrix patch is smaller,

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doi:10.1016/j.jchromb.2006.09.002

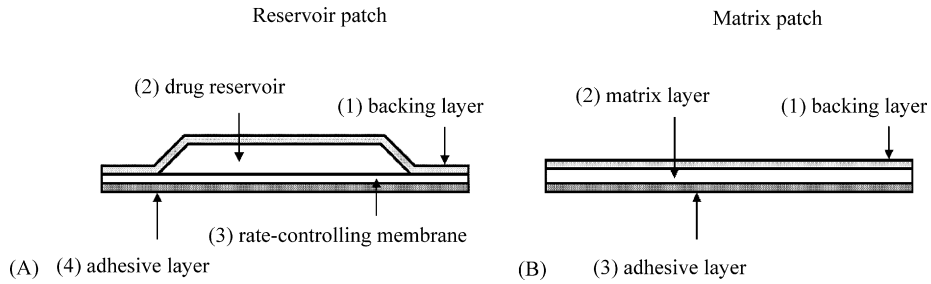

Fig. 1. Schematic representation of the functional layers in a Durogesic® reservoir type patch (A) and a matrix technology based patch (B).

thinner, more flexible and easier to apply than the reservoir patch. It offers improved adhesion and it is also associated with less skin sensitisation [6]. The active drug in Durogesic® D-Trans® is contained in a polymer matrix (Fig. 1B), rather than a gel reservoir. Fentanyl is released at a rate governed by the components in the matrix, the amount delivered being proportional to the surface area. Again four patch strengths are available, releasing fentanyl at nominal rates of 25, 50, 75 and 100 μg/h.

While there is variation in dose delivered among patients, according to the manufacturer, the nominal flux of the systems is sufficiently accurate as to allow individual titration of dosage for a given patient [7]. However, there have been reports in the literature of both limited and increased transdermal absorption of fentanyl in some individuals. Exposure to external heat sources such as electric blankets, as well as fever may enhance fentanyl absorption [3] while some authors have suggested that patients suffering from excessive sweating or certain systemic skin diseases may be at risk for limited absorption of transdermal fentanyl [8,9]. Because of potential variations among individuals, precise knowledge of the rate of absorption into the systemic circulation is important in predicting the clinical efficacy and possible toxicity of a fentanyl TTS [10]. Determination of the actual fentanyl delivery rate requires an accurate estimate of both the initial and residual fentanyl content in the transdermal system applied. However, few studies have focussed on this type of assay as a measure of actual delivered transdermal dose from reservoir type patches [10–13]. Sample preparation protocols in these studies included both water and alcohol based extractions of the TTSs in which the recovery of fentanyl was induced by incision, cutting or simple immersion of the patches. Traditional analytical techniques, such as HPLC-UV and to a less extent also RIA, have been used to quantify the amount of fentanyl recovered from new or used transdermal systems. However, in most of these studies, research focused on pharmacokinetic profiling issues, a step beyond the initial method development and validation process of the assays applied. As a result, assay validation parameters as such are scarcely reported and as a consequence, the accuracy of the resulting estimate can not always be retrieved. In addition, to our present knowledge, studies investigating the actual delivery rate in the fentanyl TTS based on the matrix patch technology are not available. Therefore, we have developed a rapid gas chromatographic–mass spectrometric (GC–MS) analytical method for the determination of residual fentanyl in used Durogesic® reservoir patches and Durogesic® D-Trans® matrix systems. Moreover, we have thoroughly validated each single step in the patch sample preparation and analysis protocol to estimate the relative contribution to systematic and random errors in the effective dose estimate. This validated analytical procedure can provide important insight in the intra- and inter-individual variations in actual rates of transdermal fentanyl delivery and by extension, in potential factors affecting this process.

2. Experimental

2.1. Chemicals and materials

Fentanyl citrate (*N*-phenyl-*N*-(1-(2-phenylethyl)-4-piperidinyl)-propanamide citrate) and the internal standard analogue $^{2}H_5$-fentanyl citrate (*N*-phenyl-*N*-(1-(2-($^{2}H_5$-phenyl)-ethyl)-4-piperidinyl)-propanamide citrate, isotopic purity >99.99% as determined by GC–MS-SIM) were kindly provided by Janssen Pharmaceutica (Beerse, Belgium). Methanol (HPLC grade) was obtained from Fisher Chemicals (Leicester, UK). Isotonic saline solution (0.85% w/v NaCl), *n*-heptane (HPLC grade) and *iso*-amylalcohol (anhydrous) were obtained from Sigma-Aldrich (Steinheim, Germany). All other chemicals were of analytical grade. Regal surface wipes (5 cm × 5 cm, 8 ply) were from Johnson & Johnson Medical Ltd. Durogesic®(reservoir) and Durogesic® D-Trans® (matrix) reference patches were from Janssen-Cilag n.v. (Berchem, Belgium). Disposable 15 ml sample tubes (16 × 100 mm), disposable 50 ml Falcon extraction tubes (30 × 150 mm) and a Heidolph Reax 2 auto shaker were supplied by VWR (Heverlee, Belgium). Merck (Darmstadt, Germany) supplied the 1 ml EXtrelut® NT1 Solid Phase Extraction columns. Autosampler vials (2 ml, crimp cap) were obtained from Macherey-Nagel (Düren, Germany).

2.2. Instrumentation and chromatographic conditions

The analyses were carried out on an Agilent 6890 series gas chromatograph equipped with an autosampler and a 5973 series mass selective detector (MSD) in electron impact (EI) mode (70 eV). A 1 μl aliquot of the sample was introduced in a splitless way onto a DB5-MS (J&W) column with a nominal length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.1 μm. A constant high purity Helium flow of 2.5 ml/min was applied through the column. The GC separation was obtained using a program with an initial oven temperature of 120 °C that was increased at a rate of 60 °C/min to a final temperature of 280 °C. The oven was held at the final temperature for an

additional 3.0 min. The injector and MS source temperature were maintained at 230 °C. The MS quadrupole temperature was held at 150 °C. The mass selective detection system was operated in the selected ion monitoring (SIM) mode. Base ion fragments occurring at *m/z* 245 for fentanyl, and *m/z* 250 for $^{2}H_5$-fentanyl were monitored and used for subsequent quantification. Individual ion dwell times were set at 50 ms for both ion fragments.

2.3. Collection of used Durogesic® patches

To demonstrate the application of the method to actual samples, a small subset of used Durogesic® patches were assayed from a number of selected cancer patients who were admitted to the palliative department of the University Hospital of Leuven (Belgium). The patients were fully informed about the procedure and the purpose of the experiment. Durogesic® patches had been applied and were removed 3 days later by the hospital staff. Patches were obtained on several occasions in a period from April to December 2000. Upon removal of the fentanyl TTS, patches were transferred to individual bags and for each patch the effective duration of application (hours) was registered. All patches were submitted to the laboratory within one week and were stored at 4 °C until analysis.

2.4. Sample preparation of Durogesic® reference and used patches

At each occasion, five new, unused Durogesic® patches with a nominal dose corresponding to that of a set of used patches, were opened and prepared as reference samples. New and used Durogesic® patches were removed from their individual bag, placed on a wipe and cut in equal pieces using small scissors. The resulting pieces had a width of 0.77 ± 0.07 cm (mean ± SD) and a length equalling the width of the original patch (i.e. 2.5–6 cm). For each patch, the pieces were transferred to individual 50-ml extraction tubes. A new wipe was used to clean the scissors and was also added to the corresponding sample tube. In the saline based protocol, each patch was extracted with 40 ml of isotonic saline and the samples were shaken in a Heidolph Reax 2 auto shaker for 16 h. Aliquots of 1 ml of extract were pipetted into a disposable 15 ml sample tube. The extracts were basified with 20 μl of 10 N NaOH and 50 μl of an internal standard solution containing $^{2}H_5$-fentanyl (120 μg/ml) was added. The samples were applied to a 1-ml EXtrelut® NT1 SPE column. After 10 min, elution was carried out using 6 ml of a mixture of *n*-heptane/*iso*-amylalcohol (98.5/1.5, v/v). One ml of the final elute was transferred to a 2 ml automatic sampler vial and analyzed. On each occasion, aliquots of 40 ml of isotonic saline were run as an analytical blank. In the methanol based protocol, Durogesic® D-Trans® matrix patches were extracted with 20 ml of methanol containing $^{2}H_5$-fentanyl (1 μg/ml). The samples were shaken in a Heidolph Reax 2 auto shaker for 15 min and 40 μl of each extract was diluted to 1 ml with methanol containing $^{2}H_5$-fentanyl (1 μg/ml) and analyzed. Sample work-up was performed in triplicate starting from the solid phase extraction in the saline base protocol and starting from the extract dilution in the methanol based procedure.

2.5. Preparation of linear regression calibrators and reference extraction solutions

Reference extraction solutions were prepared in isotonic saline corresponding to the anticipated fentanyl concentrations resulting form a quantitative extraction of new Durogesic® reservoir and Durogesic® D-Trans® matrix patches. The concentration of these reference solutions ranged from 62.5 to 250 μg fentanyl per ml of isotonic saline for the reservoir type patches and from 105 to 420 μg fentanyl/ml for the matrix patches. Linear regression calibrators ($n = 6$) were prepared in methanol and showed fentanyl concentrations of 5, 10, 20, 30, 50 and 70 μg/ml. All calibrators were fortified with $^{2}H_5$-fentanyl as an internal standard in a final concentration of 1 μg/ml. Individual fentanyl concentration levels were selected to simulate sample composition resulting from 1 ml-SPE-sample preparation of the extracted patches. Table 1 gives an overview of the initial fentanyl content in new Durogesic® patches (mg) and the corresponding anticipated concentrations (μg/ml) following extraction with 40 ml isotonic saline and subsequent 1-ml SPE-sample preparation. In this anticipation all extraction recoveries were assumed to be 100%.

2.6. Estimation of effectively delivered fentanyl transdermal dose rate and calculation of transdermal delivery efficiency

For each patch analyzed, the residual fentanyl content was determined comparing peak area ratios of fentanyl to its deuterated analogue in used patches to those found in new

Table 1
Initial fentanyl content in new Durogesic® reservoir and Durogesic® D-Trans® matrix patches (mg) and anticipated fentanyl concentrations (μg/ml) following extraction of each patch with 40 ml of isotonic saline and subsequent 1-ml SPE sample preparation

Nominal dose (μg/h)	Durogesic® reservoir type patch			Durogesic® D-Trans® matrix type patch		
	Initial fentanyl (mg)	40-ml extraction fentanyl (μg/ml saline)	1-ml SPE fentanyl (μg/ml)	Initial fentanyl (mg)	40-ml extraction fentanyl (μg/ml saline)	1-ml SPE fentanyl (μg/ml)
25	2.5	62.5	10.4	4.2	105.0	17.5
50	5.0	125.0	20.8	8.4	210.0	35.0
75	7.5	187.5	31.3	12.6	315.0	52.5
100	10.0	250.0	41.7	16.8	420.0	70.0

In this anticipation all extraction recoveries were assumed to be 100%.

Durogesic® patches of corresponding nominal doses. The difference between the initial and residual content of each patch (μg fentanyl) was assumed to be delivered to the skin depot of the patient and hence was available for subsequent absorption. The effectively delivered fentanyl transdermal dose rate (μg/h) was then estimated using the effective duration of application (h) (1). Finally, delivery efficiency (%) was expressed as the ratio of the estimated effectively delivered fentanyl dose rate to the nominal patch dose (2).

$$\text{Estimated dose rate (}\mu\text{g/h)} = \frac{\text{fentanyl content}_{\text{initial}}(\mu\text{g}) - \text{fentanyl content}_{\text{residual}}(\mu\text{g})}{\text{duration of application (h)}} \quad (1)$$

$$\text{Delivery efficiency (\%)} = \frac{\text{estimated dose rate (}\mu\text{g/h)}}{\text{nominal dose (}\mu\text{g/h)}} \times 100 \quad (2)$$

3. Results

3.1. GC–MS analysis

In the optimized saline based and methanol based protocols no significant interference was observed in analytical blank samples, and the GC–MS analytical method operated in the SIM mode was shown to be adequately selective with regard to the presence of other compounds in the Durogesic® patches. Multiple assays ($n = 10$) of the linear regression calibrators proved the analytical GC–MS method to show excellent linearity (R^2 ranging from 0.990 to 0.998) and an adequate coefficient of variation (CV, 1.9%–5.5%) was observed.

The optimization of the analytical method with regard to the limit of quantification (LOQ) was not of primary interest in this study, as the extracted new and used Durogesic® patches resulted in considerable fentanyl levels. Nevertheless, it was calculated that a 72-h application of a Durogesic® reservoir patch with the lowest nominal dose available (25 μg/h) would result in a final fentanyl concentration of 2.9 μg/ml of extract. Considering possible losses during sample processing, the target LOQ was set 1/10 of the expected concentration, i.e. 0.29 μg/ml of elute. Analysis of a fentanyl calibrator solution of 0.29 μg/ml showed an adequately detectable peak with an S/N ratio of >1500, while replicate injections ($n = 10$) revealed a coefficient of variation (CV) of 3.5% on the quantitative result. Finally, the method's linearity was demonstrated down to this LOQ concentration and an additional calibrator of 2.9 μg/ml was selected for the evaluation of the assay of used 25 μg/h patches.

3.2. Extraction recovery

Using the saline based sample preparation protocol, the extraction recovery of fentanyl from new Durogesic® reservoir and matrix type patches was determined by processing and analyzing sets of three replicate patches at different time intervals ranging from 1 to 24 h. Recovery was expressed as the percentage of the compound found in the extracted samples to that found in the anticipated reference extraction solutions. For each nominal dose level, extraction recovery was found to increase steadily as a function of time, as shown in Fig. 2. The curves were modelled into the datasets using a logarithmic function but were added only for an indication of a trend purpose. After a period of 16 h only a minor additional gain in extraction recovery was observed and in further research an extraction period of 16 h was considered to be most appropriate. Also for the matrix technology based patches, saline based extraction recoveries tended to increase as a function of time, although this increase was found to be less pronounced, except for the 25-μg/h dose level (Fig. 3). Again, curve fitting was based on a logarithmic regression type. Excepting the 25-μg/h dose level, the 16-h extraction recoveries for the matrix type of patches were at least 20% lower than for the reservoir type patches. Differences in the properties of the

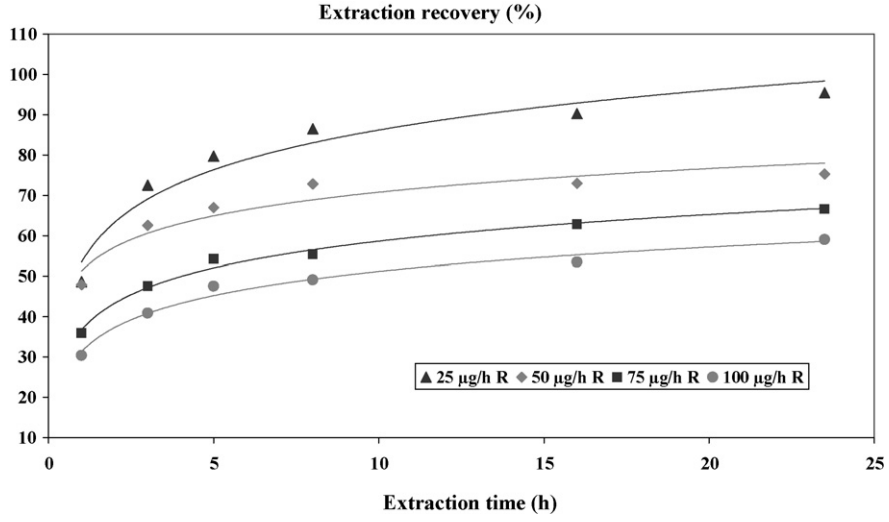

Fig. 2. The saline based extraction recovery (%) of fentanyl from new Durogesic® reservoir type patches (R) of increasing nominal dose (25, 50, 75, 100 μg/h), as a function of extraction time (h). Average recoveries ($n = 3$) are shown.

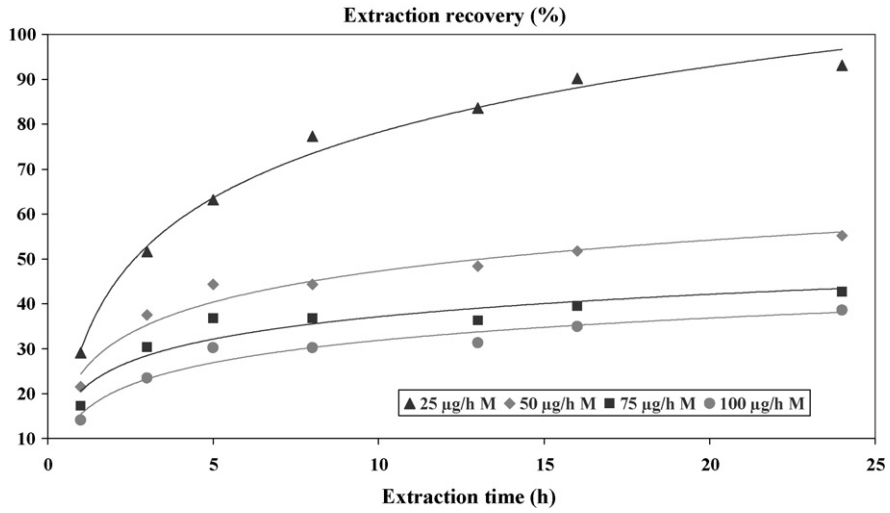

Fig. 3. The saline based extraction recovery (%) of fentanyl from new Durogesic® matrix type patches (M) of increasing nominal dose (25, 50, 75, 100 µg/h), as a function of extraction time (h). Average recoveries ($n = 3$) are shown.

typical and distinct patch technologies were hypothesized to be the basis of the observed contrasts in extraction time profiles. In accordance, dissimilarities were also observed when plotting the extraction recoveries as a function of increasing dose level of both patch systems at distinct time intervals (Fig. 4). For the reservoir type the extraction recovery tends to decrease in almost a linear way with increasing patch nominal dose rate, while for the matrix based patches a second order decrease in extraction efficiency seemed to occur.

In order to increase the extraction recovery of fentanyl from the matrix-TTS, a methanol based extraction protocol was applied for this patch type. Using this protocol, virtually quantitative recoveries of fentanyl were demonstrated for each patch nominal dose level at extraction intervals as low as 5 min (Fig. 5). The curves were modelled into the datasets using a second order polynomial function but were added only for an indication of a trend purpose. In further experiments an extraction period of 15 min was selected, since at this point recoveries tend to converge to an optimum of 100% for all nominal dose levels. Subsequent experiments applying this methanol based extraction protocol for the extraction of reservoir type patches as well, revealed the presence of aqueous components interfering at the GC–MS analysis level, and therefore, for this type of fentanyl TTS the initial saline based extraction protocol was retained.

3.3. Intra-assay and inter-assay precision

The analytical intra-assay and inter-assay precision was determined by repeated analyses of sets of five new Durogesic® patches on three distinct occasions, over a time period of approximately 3 months. Precision parameters were estimated using one-way ANOVA with the occasion of measurement as the grouping variable. Saline based assay precision data for the reservoir type fentanyl TTS are presented in Table 2. Intra-

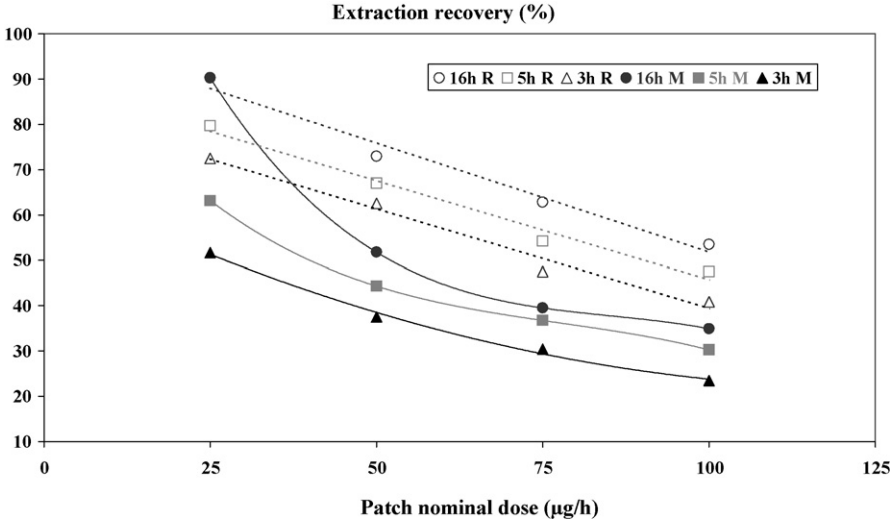

Fig. 4. The saline based extraction recovery (%) profile of fentanyl from new Durogesic® reservoir (R) and matrix (M) type patches at discrete extraction time intervals (3, 5, 16 h) as a function of increasing nominal dose (25, 50, 75, 100 µg/h). Average recoveries ($n = 3$) are shown.

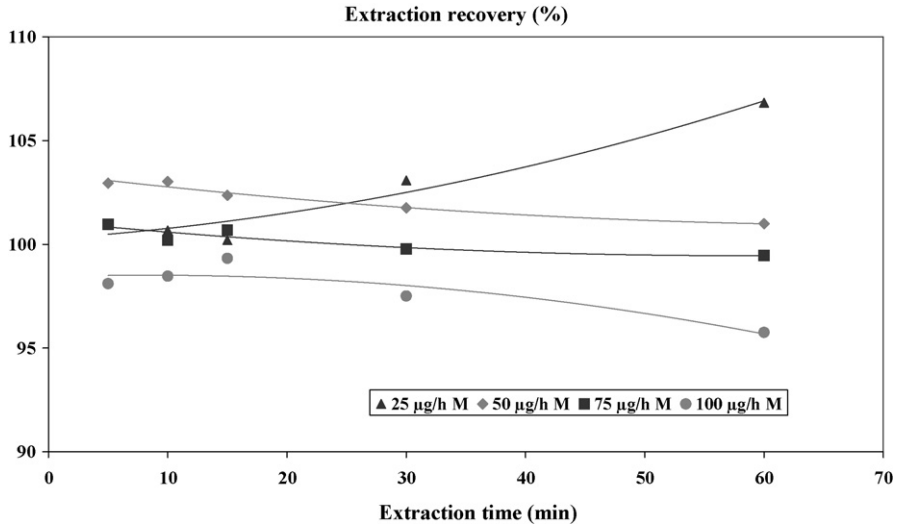

Fig. 5. The methanol based extraction recovery (%) of fentanyl from new Durogesic® matrix type patches (M) of increasing nominal dose (25, 50, 75, 100 μg/h), as a function of extraction time (min). Average recoveries ($n = 3$) are shown.

Table 2
One-way ANOVA estimates of intra-assay and inter-assay precision ($n = 5$ replicates on $k = 3$ occasions) and relative error of calculated fentanyl content from new Durogesic® reservoir type patches, using the saline based (16 h) extraction protocol

Nominal dose (μg/h)	Initial fentanyl (mg)	Intra-assay precision		Inter-assay precision		RE (%)
		Calculated fentanyl ± SD (mg)	CV (%)	Calculated fentanyl ± SD (mg)	CV (%)	
25	2.5	2.5 ± 0.05	2.0	2.5 ± 0.05	2.0	−1.6
50	5.0	4.8 ± 0.17	3.5	4.8 ± 0.17	3.5	−3.0
75	7.5	7.2 ± 0.30	4.1	7.2 ± 0.94	13.1	−4.2
100	10.0	10.5 ± 0.94	9.0	10.5 ± 0.88	8.4	4.6

SD = standard deviation, CV = coefficient of variation, and RE = relative error.

assay coefficients of variations were typically below 10%, while inter-assay precision generally showed coefficients of variations below 10%, except for the 75 μg/h nominal dose patch (13.1%). For each nominal dose, the inter-assay accuracy of the protocol was estimated by comparing the calculated initial fentanyl content with the nominal fentanyl level of the patch and was characterized by relative errors (RE) ranging from −4.2% to 4.6%.

Methanol based assay precision data for the matrix type fentanyl TTS are presented in Table 3. All intra-assay and inter-assay coefficients of variations were below 7%, while the inter-assay accuracy was characterized by relative errors (RE) ranging from −6.6% to 7.0%.

3.4. Stability of fentanyl in used Durogesic® patches upon storage at 4 °C

To estimate the stability of fentanyl during storage of used Durogesic® patches, new patches were opened as would be done prior to application, and transferred to individual bags. For each nominal dose, three patches were stored during 2 and 4 weeks and one patch was stored during 3 and 6 months to reflect reasonable and worst case storage periods of used patches. Recovery was expressed as the amount of fentanyl found in stored samples as compared to freshly opened Durogesic® patches. The results presented in Table 4 indicate that opened, stored Durogesic® patches are stable during at least 3 months as a quantitative

Table 3
One-way ANOVA estimates of intra-assay and inter-assay precision ($n = 5$ replicates on $k = 3$ occasions) and relative error of calculated fentanyl content from new Durogesic® matrix type patches, using the methanol based (15 min) extraction protocol

Nominal dose (μg/h)	Initial fentanyl (mg)	Intra-assay precision		Inter-assay precision		RE (%)
		Calculated fentanyl ± SD (mg)	CV (%)	Calculated fentanyl ± SD (mg)	CV (%)	
25	4.2	3.9 ± 0.26	6.7	3.9 ± 0.27	7.0	−6.6
50	8.4	8.0 ± 0.37	4.6	8.0 ± 0.46	5.7	−4.1
75	12.6	12.7 ± 0.60	4.7	12.7 ± 1.1	8.9	0.9
100	16.8	18.0 ± 0.75	4.2	18.0 ± 1.0	5.7	7.0

SD = standard deviation, CV = coefficient of variation, and RE = relative error.

Table 4
Recovery of fentanyl (%) from opened, unused Durogesic® reservoir type patches stored during two and four weeks ($n = 3$) and during three and six months ($n = 1$)

Nominal dose (μg/h)	Recovery ± SD (%) upon storage			
	2 weeks	4 weeks	3 months	6 months
25	104 ± 4	105 ± 0.1	103	91
50	97 ± 4	100 ± 4	100	78
75	102 ± 8	99 ± 4	113	77
100	97 ± 3	100 ± 2	102	77

Patches were assayed with the saline based protocol. SD = standard deviation.

recovery of fentanyl is demonstrated. Upon storage during 6 months a slight to considerable loss of fentanyl seems to occur, yielding recoveries of 77%–91%.

3.5. Application

The analytical method described was developed to estimate effective fentanyl transdermal absorption through the application of Durogesic® patches in a large study involving over 60 patients of the palliative department of the University Hospital. The presentation of the results of the assay of over 500 patches and the discussion on identified factors influencing the effective delivery of transdermal fentanyl, fall beyond the scope of this method development study and will be presented in detail elsewhere. However, the application in-the-field of the presented saline based analytical protocol is illustrated by the analysis of a sample subset of 57 Durogesic® patches obtained from four palliative patients, each individually treated with a stable transdermal fentanyl dose of either 25, 50, 75 or 100 μg/h during a considerable period of time. For each individual patch, the effectively delivered dose rate was estimated as well as the delivery efficiency and for both parameters a mean value was calculated. For each patient, characteristics are summarized in Table 5 and time profiles of estimated effectively delivered dose rate (μg/h) are shown in Fig. 6. These results indicate that the developed analytical procedure is easily applicable to monitor residual fentanyl and to estimate effective transdermal fentanyl delivery. Although in this illustration the number of assayed samples was small, insight was gained in the potential magnitude of intra-individual variations over time of estimated effectively delivered fentanyl through the use of transdermal Durogesic® fentanyl patches of available nominal dose rates.

4. Discussion

Until very recently, few studies involving a very limited number of samples have focussed on the assay of residual fentanyl in used fentanyl TTS as a measure of actual delivered transdermal dose. More importantly, in these studies, a thorough validation

Table 5
Patient characteristics and results of the analysis of a number of applied Durogesic® reservoir type patches, expressed as estimated dose rate (μg/h) and delivery efficiency (%) ± SD and intra-patient coefficient of variation (CV) on the estimated parameters

Patient	Sex	Nominal dose (μg/h)	Application site[a]	No. of patches analyzed	Estimated dose (μg/h) ± SD	Delivery efficiency (%) ± SD	CV (%)
1	F	25	Arm	16	29.3 ± 4.0	117.3 ± 16.1	13.8
2	M	50	Arm	14	46.3 ± 3.6	92.6 ± 7.2	7.8
3	F	75	Torso	9	74.6 ± 4.1	99.5 ± 5.5	5.5
4	M	100	Arm/Leg	18	86.6 ± 12.2	95.9 ± 18.6	14.1

[a] Predominant site of application of the analyzed Durogesic® patches.

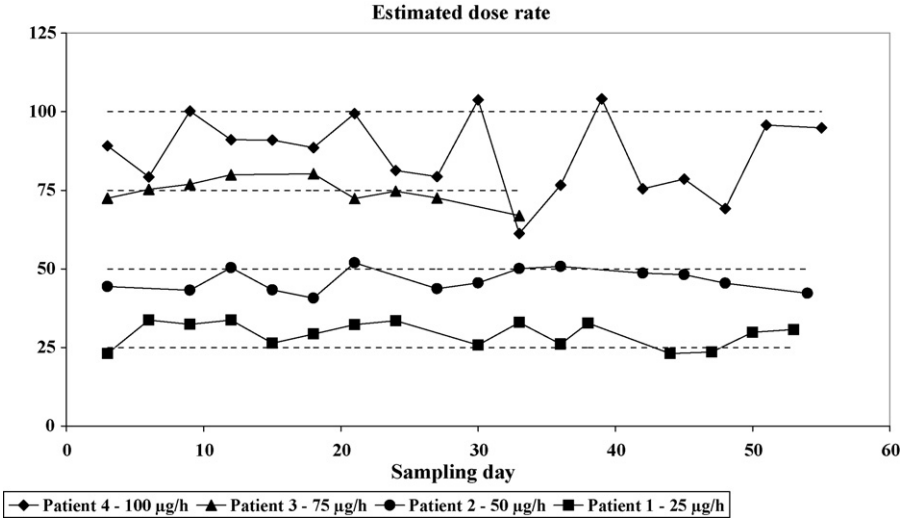

Fig. 6. Time profile of estimated transdermal dose rate (μg/h) in four selected palliative patients treated with Durogesic® reservoir type patches at the available nominal dose levels (25, 50, 75, 100 μg/h).

of all steps in the applied sample preparation protocol and the analytical methodology is generally lacking, mainly because the research performed comprises a pharmacological or toxicological approach, rather than a method development issue.

Varvel et al. [10], in 1989, was the first to report a study in which residual fentanyl content of spent transdermal reservoir patches was determined to calculate the actual rate of absorption and the systemic bioavailability of transdermally administered fentanyl. Following removal of eight 100-μg/h TTSs from patients (at 24 h), residual fentanyl was extracted from each system with 50:50 acetonitrile:0.02 N sulfuric acid and was measured by an HPLC technique. The residual fentanyl content in the used TTSs was then compared with the average initial content of 10 unused 100-μg/h transdermal fentanyl systems. Analysis of the latter systems showed the mean and standard deviation of initial fentanyl content to be 10.2 ± 0.4 mg. These results are comparable with the data obtained in our study, in which the initial fentanyl content of fifteen 100-μg/h patches averaged 10.5 ± 0.88 mg.

In the early 1990s, Portenoy et al. [11] used the same extraction procedure and HPLC technique as described by Varvel et al. [10] to measure residual fentanyl in eight used reservoir type 100-μg/h TTSs to investigate repeated dose pharmacokinetics of this system. No further method details or validation parameters were reported except for "assay procedures were validated previously by this (Alza Corporation) laboratory".

In the mid 1990s, Marquardt et al. [12] used a fentanyl-specific radioimmunoassay (RIA) for the determination of residual fentanyl in five 25-μg/h and four 100-μg/h patches, which had been cut up and extracted with methanol. As a control, one unused 2.5-mg patch was analyzed and showed a 94% recovery of fentanyl. The assay, which was dedicated to warrant against potential abuse and misuse of spent fentanyl patches, was reported to have a coefficient of variation of 3.3%–4.0% over the range studied. Both analytical parameters are consistent with the data reported in our study, comprising a extraction recovery approaching 100% and an intra-assay coefficient of variation of 4.2%–6.7%.

Most recently, a study by Solassol et al. [13] focused on the inter- and intra-individual variability in transdermal fentanyl absorption in cancer patients. Prior to analysis, the used patches were incised and fentanyl was extracted by mechanical shaking with 50 ml of 1 M hydrochloric acid during 24 h. The necessary time to achieve total fentanyl dissolution from the patches was determined by the establishment of a fentanyl recovery versus time profile of three unused 75-μg/h patches. Although the dissolution progress for this patch dose was well documented, the results of our study strongly indicate that the extraction time profile and resulting recoveries may differ for each patch nominal dose level. Intra-day ($n = 6$) and inter-day ($n = 11$) precision and accuracy of the reported HPLC-UV method was assessed by analyzing QC samples, and was reported to be 4.9%–9.1% precision and 99.1%–104.6% accuracy. Although it is not clear whether these data are based on the repetitive analysis of reference (i.e. unused) QC-patches or rather fentanyl QC calibrator solutions, precision outcomes seemed to be comparable to those found in our study (2.0%–9.0% and 2.0%–13.1%).

Calculation of the actual dose rate of transdermal delivered fentanyl requires an accurate estimate of both the initial and residual fentanyl content in the TTS. Obviously, it is not possible to determine the fentanyl content in a particular system prior to its application on the patient and therefore reference must be made to the analysis of new, unused patches of equal nominal dose. Although, as a consequence, there is some error inherent in the estimation of the delivered dose, this study has shown that the initial fentanyl content in both new reservoir and matrix type patches can be assayed with high reproducibility and excellent accuracy, with coefficients of variation and relative errors generally below 10%. However, to accurately estimate the residual fentanyl level in used TTSs and the initial fentanyl content in new patches, a precise knowledge of the extraction recovery for each patch type is imperative. The results of our study strongly indicate that in the saline based extraction protocol, extraction efficiency versus time profiles may have similar shapes for distinct nominal doses but may result in substantially different recoveries at equilibrium. The dependency of this equilibrium recovery on the initial dose rate of the patch was hypothesized to be due to a fixed amount of fentanyl being unavailable for extraction. This amount of fentanyl thus remaining in the patch clearly increased with increasing patch dose and increasing patch size, resulting in lower recoveries for the highest patch doses. It is therefore considered essential that reference is made to the analytical result of a batch of unused fentanyl TTSs of equal nominal dose, subjected to exactly the same extraction conditions as each set of used patches being assayed for residual fentanyl content. As a consequence, used fentanyl TTSs are most likely to be analysed batch-wise, possibly following a certain period of time during which patches were collected and stored. Our study evaluated the stability of the reservoir type patches during cooled storage (4 °C) and showed that opened, unused fentanyl TTSs can be stored up to 3 months without significant loss.

The optimized and validated GC–MS analytical protocol was successfully applied for the determination of residual fentanyl in over 500 used reservoir type patches after removal from palliative patients. The results of the assay of a subset of 57 patches obtained from four patients provided a clear illustration of the magnitude of intra-individual variation in estimated transdermal fentanyl dose observed over time (Fig. 6). Although for each patient studied the effectively delivered fentanyl dose rate appeared to fluctuate around the specified nominal dose level, considerable intra-individual variations were observed, characterized with a 'within-patient' coefficient of variation of 5.5%–14.1%. Assay results indicating an increase in estimated fentanyl transdermal delivery could be associated with factors affecting systemic or local body temperature (fever, external heat sources) although some caution in the interpretation of the assay outcome is needed. Indeed, an overestimation of the effectively delivered transdermal rate might result from an underestimation of the residual fentanyl content of the patch, which in turn might be due to compound loss during storage or sample preparation. In contrast, analytical results revealing estimated fentanyl transdermal rates lower than the specified nominal dose level, should draw the attention of the hospital staff to the correct application of the patches or other patient related conditions, possibly

lowering transdermal fentanyl absorption efficiency, like sweating. Therefore, the presented analytical method could prove an excellent usefulness by its potential to identify 'slow absorbers' or 'poor responders' to transdermal fentanyl therapy.

Acknowledgements

The authors are grateful for the excellent technical assistance by Peter Collaerts and Karin Vranckx and wish to acknowledge the kind donation of the fentanyl and penta-deuterated fentanyl by Janssen Pharmaceutica (Beerse, Belgium). Special thanks are due to Katrien Poels for closely reading through this manuscript.

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