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Journal of Chromatography B, 857 (2007) 83–91
JOURNAL OF CHROMATOGRAPHY B
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Method to determine stability and recovery of carboprost and misoprostol in infusion preparations

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Received 13 October 2006; accepted 1 July 2007
Available online 13 July 2007

Abstract

The two synthetic prostaglandin analogues, carboprost and misoprostol, are used extensively in obstetric and gynaecological practice. Our recent research of these compounds' use for intra-umbilical injection to treat adherent placenta necessitated their storage in solution for 3–4 days. This raised concerns over the stability and applied dosage in the in-house infusion preparations. It requires various pharmacological preparations before administration in clinical practice. We used LCMS to develop a simultaneous, valid, fast and simple method to assess the stability and recovery of their in-house preparations in different conditions. The linearity between 0–40 μg/ml was above 0.995. The reproducibility (CV) was within 5.2%. The limit of quantitation of the method for both compounds is about 2 μg/ml. The accuracy of both compounds from 0.4–40 μg/ml is 96.4–104.3% while the precision is 0.4–7.4%. The recoveries of carboprost in the infusion were from 100.3 ± 4.0 to 102.4 ± 1.6% and that of misoprostol in Cytotec tablet was from 44.9 ± 3.5 to 50.0 ± 5.0% in water and saline at 4 °C and room temperature. No interference was found from the matrix and between the tested compounds. The compounds were basically stable for 6 days in water and in saline, whether they were stored at 4 °C or at room temperature. However, only half of the dosage of misoprostol was recovered in the solution. Therefore, misoprostol dosage should be adjusted before clinical application.

Keywords: Carboprost; Misoprostol; Stability; Recovery

1. Introduction

Carboprost, a 15 methyl $F_{2\alpha}$ prostaglandin (Fig. 1), marketed as Hemabate™, is widely recommended as a treatment for post-partum haemorrhage. Misoprostol, a prostaglandin E1 analogue, marketed as Cytotec™ in tablet form, has been widely used in obstetric and gynaecological practice to enhance uterine tone and contractility [1–4], despite only having FDA approval to treat and prevent intestinal ulcers. The reason for this is the large difference in the market prices and availability of these two drugs: misoprostol is marketed as an over-the-counter drug in Hong Kong at less than US$1 for 200 mcg whereas carboprost is available only through restricted tender to major hospitals at more than US$100 per 250 mcg injection. There is no official pharmaceutical preparation of misoprostol specifically approved for gynaecological use. Practitioners therefore tend to make up their own trial preparations. Misoprostol is commonly administered orally or vaginally using suppositories or tablet fragments [1–3]. Alternatively, the tablets can be reconstituted into a gel suspension, but this significantly reduces its efficacy [5]. In order to facilitate a recent research project on the prevention of manual removal of retained placaenta (MROP), uterotonic solutions, including misoprostol, were prepared in advance and stored for up to 4 days before umbilical vein injection [6]. Concerns have been raised in a number of studies as to the accuracy of dosage application levels of misoprostol in such preparations [1,7].

Variations in recovery and stability of misoprostol may explain differences in its reported efficacy. However, its recovery and stability in solution have not been formally studied. Moreover, these properties have not been compared with commonly used and approved carboprost injection.

Since prostaglandins have no chromophore, it is not practical to analyze them by UV or florescence detection. Some studies have used a low UV range for misoprostol detection [8,9], but the

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doi:10.1016/j.jchromb.2007.07.016

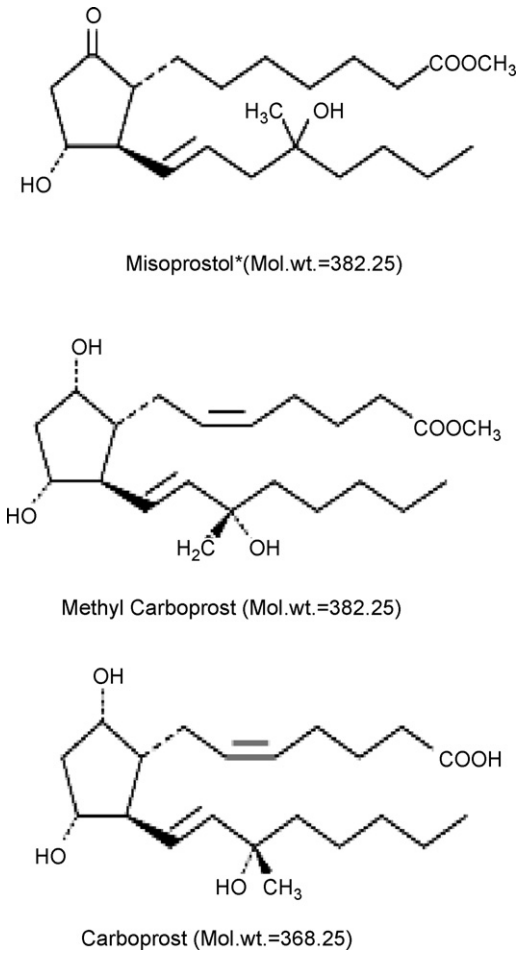

Fig. 1. Structures of misoprostol, internal standard methyl carboprost and carboprost. (*) No steric structure is shown for misoprostol as it is supplied as racemic (mirror image) isomers (±)-15-deoxy-(16RS)-16-hydroxy-16-methylprostaglandin E1 methyl ester mixture.

determinations were seriously influenced by solvent effect. Most studies have attempted to use mass spectrometry for detection of these prostaglandins. GC-MS has been used for misoprostol analysis in milk and serum [10]; however, this required triple derivatization by pentafluorobenzyl (PFB), pentafluorobenzyl oxime (PFBO) and SilPrep with many sample cleanup and evaporation procedures. In addition, syn-/anti-PFBO isomers were obtained which made the determination complicated. LCMS has been employed with positive or negative ionization mode [7,11], but neither study validated a method for simultaneous determination of misoprostol and carboprost. Unlike other quantitative spectroscopic methods, the reproducibility of LCMS is often low due to ion suppression effect and spray stability. In addition, in order to compare the stability of both drugs, it is essential to evaluate the compounds with the same studying period, i.e. from the start of the experiment to the sample assay. Therefore, it is better to apply a simultaneous method with internal standard to facilitate precise comparison and to increase the measurement throughput. To meet our needs for the comparison study, we have developed and validated a method for simultaneous determination of misoprostol and carboprost levels in infusion solutions. We also compared their stability in saline and in water preparations stored either at room temperature or 4 °C.

2. Experiment

2.1. Material

LCMS-grade methanol and acetonitrile were obtained from RDH (Seezle, Germany). TFA was obtained from BDH (Poole, England). Misoprostol, carboprost and methyl carboprost standards were obtained from Cayman (MI, USA). Hemabate™ sterile solution (carboprost tromethamine) was obtained from Pharmacia (Michigan, USA). Cytotec™ tablets (200 μg misoprostol) were obtained from Pharmacia (Milton Keynes, UK). Microcrystalline cellulose and hydroxypropyl methylcellulose (HPMC) were obtained from Fluka (Buchs, Switzerland). Hydrogenated castor oil was purchased from Henkel Corporation (CA, US). Sodium starch glycolate was obtained from CarboMer (CA, USA). All other reagents used were in their highest grade.

2.2. Instrumentation

Agilent 1100 series capillary liquid chromatography (Palo Alto, CA) equipped with degasser, capillary pump, thermostatic column compartment and μWPS thermostatic autosampler. Micromass Q-Tof micro mass spectrometry (Milford, MA) equipped with electrospray ionization and atmosphere pressure chemical ionization probe. Columns included Supelco Suplex PKB 250 mm × 2.1 mm × 5 μm in conjunction with guard column 20 mm × 2.1 mm × 5 μm.

2.3. Standard preparation

Ten microlitre of 10 mg/ml of standard stock misoprostol and carboprost solutions were diluted to 100 μg/ml standard solutions with ethanol. Internal standard, 1 mg of methyl carboprost, was dissolved into 100 μl of ethanol as stock solution, and then 10 μl of the solution was diluted to 100 μg/ml with water extract of placebo powder. All standard solutions were stored at −20 °C. These solutions are claimed by the supplier to be stable for 1 year under this condition.

Standard working solutions were freshly prepared daily. The mixtures of the standards were prepared as 0, 0.4, 2, 4, 10, 20, 30 and 40 μg/ml in 30% acetonitrile and water extract of placebo powder (1:1) with internal standard at 20 μg/ml.

2.4. Placebo powder and blank water extract of placebo powder

A tablet weight (0.2 g) of placebo powder was prepared from the mixture of 2 mg hydrogenated castor oil, 10 mg sodium starch glycolate, 168 mg microcrystalline cellulose and 20 mg hydroxypropyl methylcellulose [9,12,13]. Blank water extract was prepared by mixing, homogenizing and vortexing 2 ml of water with placebo powder for 5 min. After centrifugation at

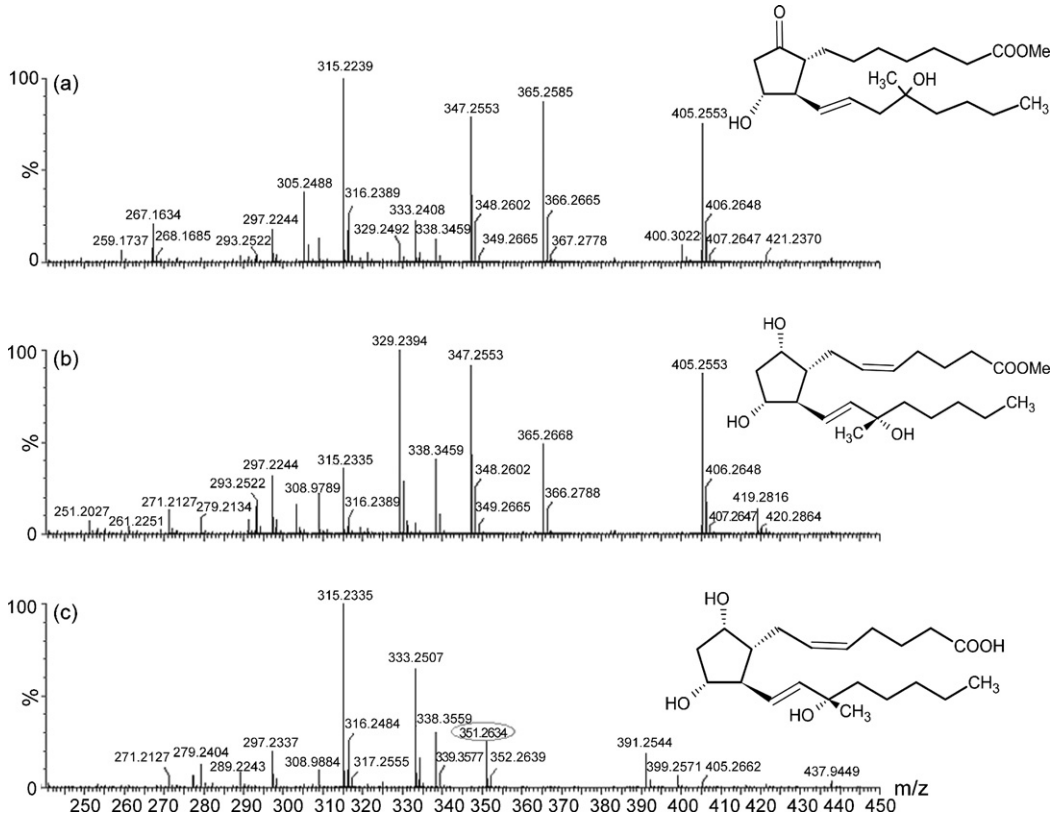

Fig. 2. Full spectrum of 20 μg/ml (a) misoprostol, (b) methyl carboprost and (c) carboprost. The circle indicates the mass selected for exact mass quantification of carboprost.

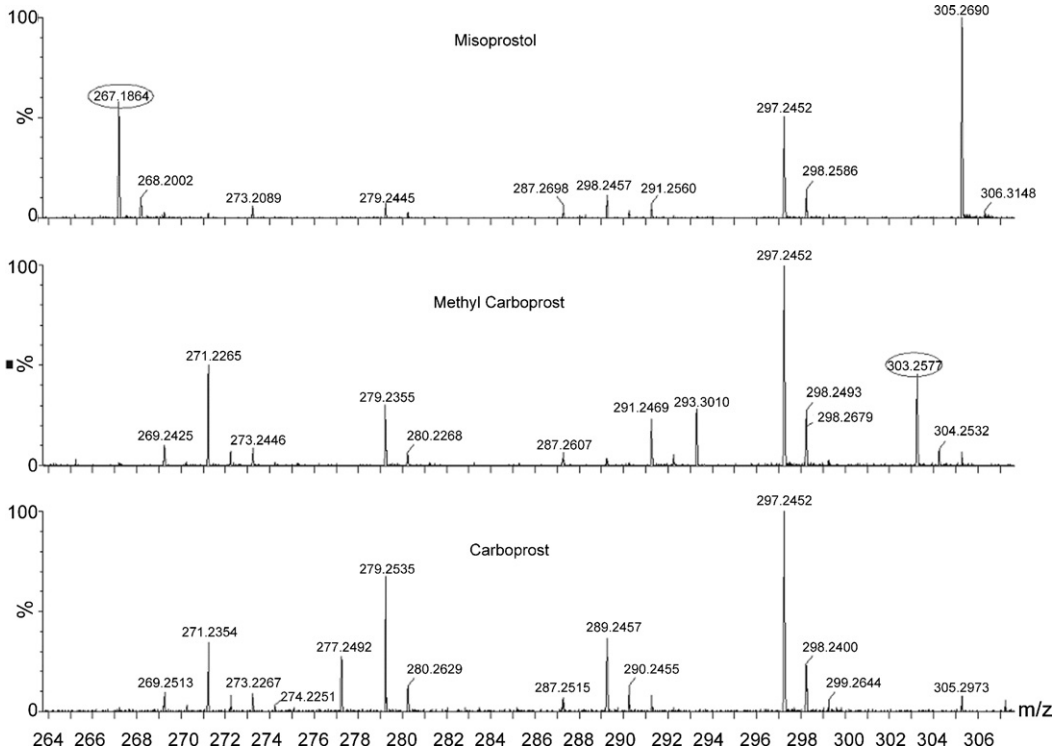

Fig. 3. Enlarged spectrum of misoprostol, methyl carboprost and carboprost show the minor specific fragments. The circles indicate the masses selected for quantification of misoprostol and methyl carboprost.

4500 rpm for 3 min, the blank water extract was obtained from the supernatant.

2.5. Sample preparation and stability test

Single Cytotec™, 200 μg misoprostol tablet, was powdered, homogenized and vortexed either in 2 ml of water or normal saline (0.9% NaCl) for 5 min. After centrifugation at 4500 rpm for 3 min, the clear supernatant was pipetted and stored at either room temperature or 4 °C in amber glass vials for 6 days. The process was repeated in triplicate.

A preliminary dissolution test was performed for the powdered misoprostol tablets over 40 min of shaking. Sample preparation was performed as described above. Twenty microlitre of the supernatant was sampled after every 5 min of shaking. The maximum dissolution of misoprostol was achieved in the initial 5 min.

One millilitre of sterile Hemabate™ solution containing 250 μg/ml carboprost was mixed with 1 ml of water or saline and stored either at room temperature or 4 °C in amber glass vial for 6 days. The procedure was repeated in triplicate.

Ten microlitre of the two preparations obtained daily (Day 0–Day 6) from each of the samples was mixed with 10 μl of 100 μg/ml internal standard solution, methyl carboprost and 20 μl of 30% acetonitrile. Eight microlitre of the final mixture was subsequently injected into HPLC.

2.6. LCMS analysis

LC separation was performed on Supelco Suplex PKB 250 mm × 2.1 mm × 5 μm with guard column 20 mm × 2.1 mm × 5 μm at 0.35 ml/min in isocratic elution at 40 °C. Mobile phase constituted methanol/acetonitrile/H_2O/TFA (50:15:35:0.05%, v/v). Positive electrospray ionization was used at 4500 V and 800 l/h nitrogen desolvation gas. The cone gas was 40 l/h and cone voltage was 30 V. The source temperature was 120 °C and the desolvation temperature was 350 °C. Manual pusher was set with cycling time at 22 μs. The optimum scanning range was set from 50 to 450 *m/z*. The exact mass method was used for integration and internal standard method was used for quantification. The exact mass fragment around 303.2577 ± 0.15 *m/z* was used for the integration of methyl carboprost, 267.1864 ± 0.15 *m/z* for misoprostol and 351.2634 ± 0.15 *m/z* for carboprost (Figs. 2–3) and (Table 1). Although there is always a very slight fluctuation of mass measurement, manual identification of the masses and smoothing of data were employed before the integration was performed.

2.7. Validation

The limit of quantitation (LOQ) of the method is assigned as the lowest concentration at which the co-efficient of variation (CV) and accuracy are within 15% of nominal standard concentration. It was assessed by spiking misoprostol and carboprost into a water extract of placebo powder at concentrations of 0.04, 0.2, 0.4, 2 and 4 μg/ml. The solutions were mixed with internal standard and 30% acetonitrile as described in sample preparation. The concentrations of the compounds were determined by LCMS. The concentrations were calculated from the calibration curve and multiplied by dilution factor 5. This exercise was repeated five times. Blank water extract of placebo powder was also used to demonstrate that no interference peaks appeared. Total ion chromatography (TIC) of the blank placebo extract was used to show that matrix peaks are eluted earlier than those of prostaglandins.

Table 1
Major fragments in carboprost, misoprostol and methyl caroprost

Compound	Molecular weight	*m/z*	Ion
Carboprost	368.25	391.25	$[M+Na]^+$
		351.26	$[M-OH]^+$
		333.25	$[M-OH-H_2O]^+$
		316.25	$[M-2OH-H_2O]^+$
		315.23	$[M-OH-2H_2O]^+$
Misoprostol	382.25	405.26	$[M+Na]^+$
		365.26	$[M-OH]^+$
		347.26	$[M-OH-H_2O]^+$
		315.23	$[M-2H_2O-OCH_3]^+$
		267.16	$[M-C_7H_{15}O]^+$
Methyl carboprost	382.25	405.26	$[M+Na]^+$
		365.26	$[M-OH]^+$
		347.26	$[M-OH-H_2O]^+$
		329.25	$[M-OH-2H_2O]^+$
		315.23	$[M-2H_2O-OCH_3]^+$
		293.25	$[M-H_2O-C_5H_{11}]^+$

The recovery was assessed by mixing 100, 200 and 300 μg of misoprostol into placebo powder (corresponding to 50, 100 and 150% of the labeled dose of Cytotec). The powder was extracted by 2 ml of water or saline, mixed with carboprost at 50, 100 and 150% of the labeled dose and 30% acetonitrile as described for sample preparation. The recovered concentrations of misoprostol and carboprost were calculated from the standard curve.

Accuracy and precision at each concentration was determined by spiking misoprostol and carboprost into blank water extract from placebo powder and 30% acetonitrile (1:1) at concentrations of 0, 0.4, 2, 4, 10, 20, 30 and 40 μg/ml with 20 μg/ml internal standards as described in standard preparation. The preparation was repeated for five times. The concentrations were evaluated from the standard calibration curve.

Stability of misoprostol during sample preparation was verified as follows: two samples, each containing 200 μg of misoprostol mixed with placebo powder, were processed as described in sample preparation but, whilst one was extracted for 5 min, the other was extracted for 10 min. Another two 200-μg misoprostol samples were mixed with 2 ml water extract of placebo powder and processed in the same way, one with extraction for 5 min and the other for 10 min. This test was repeated for three times.

Reproducibility was assessed by repeating the recovery procedure on Cytotec tablet and Carboprost injection four times with water and saline.

Selectivity was performed by spiking each individual compound into 30% acetonitrile/placebo powder extract mixture at 40 μg/ml. The three channels used to monitor the three compounds were applied to verify whether any interference peak appeared in the other channels at this concentration.

Linearity was assessed in a similar fashion to recovery but with spiked concentrations at 0, 4, 10, 20, 30 and 40 μg/ml of misoprostol and carboprost assuming 100% recovery.

2.8. Data analysis

Statistical analysis for recovery comparison was performed by Student *t*-test using Excel.

2.9. Criteria of stability

Stability of the drugs was evaluated by the percentage of the initial concentration recovering at each time interval. Stability was defined as the concentration not less than 90% of the initial value [14,15].

3. Results

Carboprost was well separated from misoprostol and internal standard, methyl carboprost. The elution of methyl carboprost and misoprostol could not be separated but could be differentiated by their specific ions: 303.2 for methyl carboprost and 267.2 for misoprostol (Figs. 2 and 3). The fragments of methyl carboprost and misoprostol were similar, although their relative abundances were different. Minor but unique fragments were shown at 303.2 for methyl carboprost and 267.2 for misoprostol (Fig. 3). The fragmentation was explained in Table 1 but the fragmentation of the unique fragment could not be explained.

The limits of quantitation of the method were about 2 μg/ml for both carboprost and misoprostol (Table 2,Fig. 4). Chromatograms of placebo powder extract indicate all matrix peaks eluted earlier than the prostaglandins and no interference peak

Table 2
Limit of quantitation (LOQ) of the method of misoprostol and carboprost was determined from 0.04–4 μg/ml in water extract of placebo powder

Concentration of water extract (μg/ml)	Misoprostol ($n=5$)		Carboprost ($n=5$)	
	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
4	88.34	4.13	105.55	5.45
2	103.60	12.75	86.84	11.56
0.4	–	–	–	–
0.2	–	–	–	–
0.04	–	–	–	–

The LOQs of both compounds are about 2 μg/ml. Five repeating samples processing were used for the evaluation at each concentration; (–), undetectable.

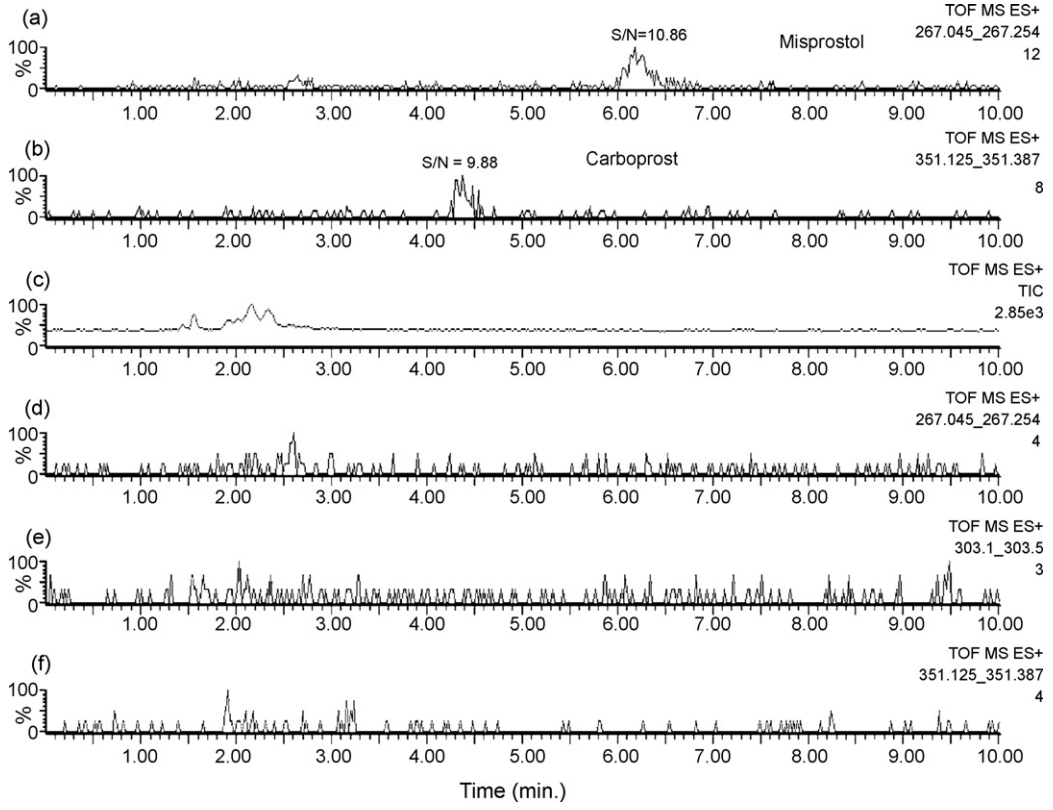

Fig. 4. Chromatogram shows the LOQ of (a) misoprostol and (b) carboprost at 2 μg/ml in water extracts of placebo powders and their S/N ratios. The chromatograms (c)–(f) show water extract of placebo powder does not contain any interference to misoprostol, carboprost and internal standard, methyl carboprost at their retention times. The number in the second row of the right side in each chromatogram describes the channel of mass range used for integration. The number in the third row of the right side in each chromatogram indicates the absolute abundance of the largest peak in each chromatogram. TIC means total ion chromatogram. The chromatograms have not been smoothed for integration.

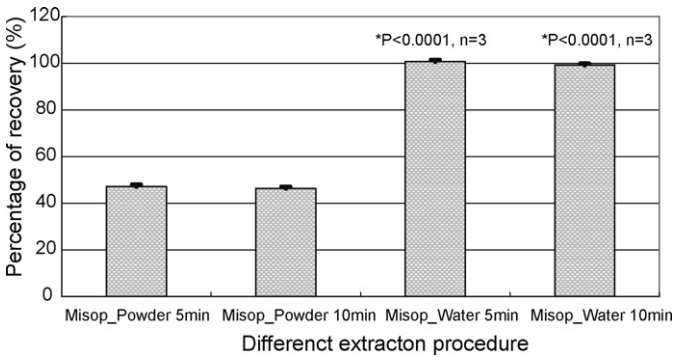

Fig. 5. Compare misoprostol stability during extraction in water extract of placebo powder and placebo powder in 5 and 10 min processing time. This test was performed in triplicate. It shows misoprostol was recovered significantly higher from water extract than from placebo powder. No significant difference in longer extraction time proves misoprostol is entrapped in the cellulose gel paste but not degraded during the extraction process. Also, almost 100% recovery of misoprostol in water extract proves misoprostol is soluble in water but could be partitioned to cellulose paste during extraction. The error bar shows the standard derivation of recovery. (*) Significant difference found by *t*-test comparing misoprostol recovery from water extract and powder extract at the same length of extraction time.

appeared from the placebo powder extract. Co-efficient of variation of five replicates of carboprost was 11.56% and of misoprostol was 12.75% at this concentration, while the accuracy was 86.84% and 103.6%, respectively.

The average recovery of carboprost was 100.3 ± 4.0 from injection solution in the 12 samples in water and 102.4 ± 1.6 (mean ± S.D.) in the 12 samples in saline. The average recovery of misoprostol from tablet powder was $44.9 \pm 3.5\%$ in the 12 samples in water and $50.0 \pm 5.0\%$ (mean ± S.D.) in the 12 samples in saline. The percentage of recovery of misoprostol from water extract after 5 and 10 min extraction was 100.7 ± 0.78 and $99.2 \pm 0.6\%$ (mean ± S.D.) while that from placebo powder at these extraction times was 47.2 ± 0.8 and $46.4 \pm 0.7\%$ (mean ± S.D.) (Fig. 5). The accuracy of the LCMS analysis over the range 0.4–40 μg/ml was 99.2–104.3% for misoprostol and 96.4–102.8% for carboprost. The precision (CV%) for misoprostol was 0.7–7.4% and for carboprost was 0.4–7.4% (Table 3).

Table 3
Accuracy and precision were determined by repeating analysis of five samples at each concentration

Concentration (μg/ml)	Misoprostol ($n = 5$)		Carboprost ($n = 5$)	
	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
0	–	–	–	–
0.4	104.32	7.45	96.44	7.36
2	102.30	3.12	97.62	4.23
4	99.23	0.72	98.86	4.84
10	102.70	1.04	102.81	0.86
20	98.77	1.21	101.21	1.45
30	99.42	1.83	102.21	0.38
40	101.65	2.25	98.57	2.05

Each concentration was determined from the calibration curve; (–) cannot be determined.

The reproducibility (CV%) of the method in four replicates was from 1.0 to 4.8% for misoprostol and from 1.35 to 5.2% for carboprost.

The linearity of the calibration standards of 0, 0.4, 2, 4, 10, 20, 30 and 40 μg/ml was above 0.995.

When stored at 4 °C the recovery of carboprost solution mixed with water and normal saline was 106.9 ± 4.1 and $106.9 \pm 3.0\%$, whereas the recovery of misoprostol from tablet to water and normal saline was only 56.1 ± 0.9 and $39.3 \pm 2.5\%$, respectively. When stored at room temperature the recovery of carboprost solution mixed with water and normal saline was 100.0 ± 2.8 and $104.2 \pm 3.0\%$, whereas the recovery of misoprostol in tablet powder to water and normal saline was 49.3 ± 2.2 and $54.3 \pm 0.7\%$, respectively.

No interference peaks were found in the channels other than the expected compounds (Fig. 6).

The drugs were stable after dissolving in water or saline at room temperature or 4 °C according to the criteria of stability. Only carboprost in water at 4 °C was noted to decrease slightly from Day 3 to about 90% of its initial concentration (Fig. 7).

4. Discussion

We used Q-Tof to quantify the compounds. Although Q-Tof is a scanning instrument mainly used for identification with a narrower dynamic range than the SIM in triple quadrupole, the instrument is still capable of quantifying the concentration range in this study. In fact, with the dead time correction, it can determine the compound in three-order range. In addition, the detection limit of Q-Tof is often not as low as that of triple quadrupole using SIM. We used exact mass for quantification to eliminate almost all the interferences. Detection limits were achieved at the μg/ml level even when minor peaks were chosen for quantification.

The parent ions of the prostaglandin analogues, 383.25 for methyl carboprost and misoprostol and 369.25 for carboprost, tend to associate with sodium ions to form sodium adducted compounds, so the abundances of the parent ions were low. Also, the compounds were fragmented even at 5–10 V cone voltage. Therefore, the parent ions were not selected for quantification. The sodium adducts were also not selected because of the great variation of abundance. The abundance of adducted ions depends on many factors such as the presence of sodium ion impurities at variable concentrations in the mobile phase, the competitive interactions with other additives and concentration of analytes. As a result, a higher cone voltage of 30 V was applied to induce in-source dissociation. The advantage of inducing fragmentation is not only to produce comparative stable fragmentation abundance but also to improve the selectivity of the method. Considering the molecular weights, structures and fragments of misoprostol and methyl carboprost were very similar, we used fragment ions instead of parent ions to provide a more reliable quantification. We used in-source fragmentation instead of fragmentation inside the collision cell as in MSMS mode to maximize the amount of fragmented ions infusing into the mass spectrometer. It, therefore, improves the sensitivity for the minor fragments detection.

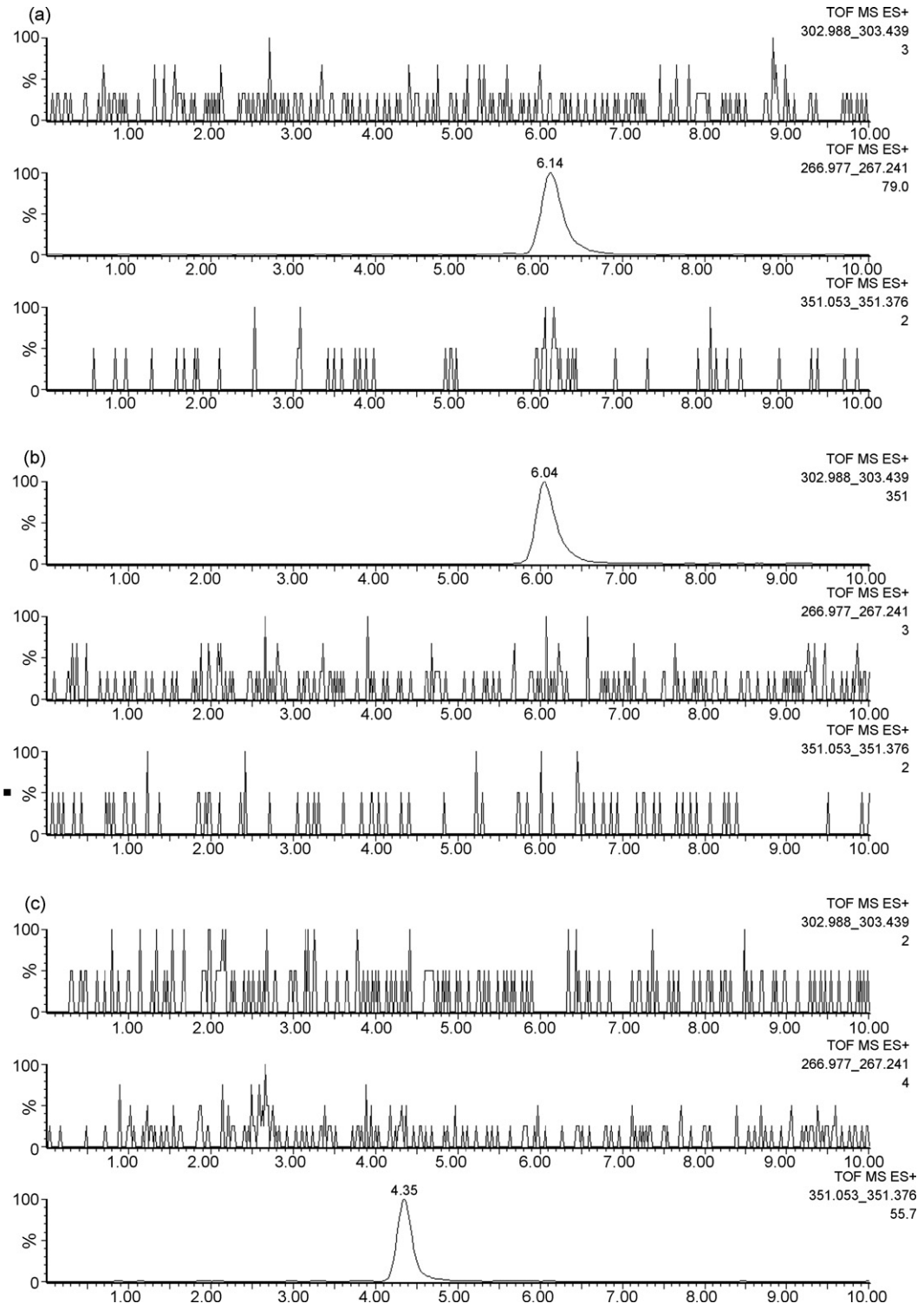

Fig. 6. Chromatogram of 40 μg/ml of (a) misoprostol, (b) methyl carboprost, (c) carboprost in blank placebo powder extract monitoring in all channels. The second row in the right hand side of each chromatogram indicates the mass range used for integration while the third row describes the absolute abundance of the largest response in the chromatogram. They showed no other interference peaks was found. Since no obvious characteristic misoprostol and methyl carboprost spectral mass peaks were found in the retention times of misoprotol and methyl carboprost, the *m/z* value is artificially assigned with reference to the *m/z* value of misoprostol and methyl carboprost in blank placebo powder extract.

We selected methyl carboprost as an internal standard because it was commercially available and its structure is very similar to both misoprostol and carboprost. Our chromatographic condition was able to separate carboprost from the other two compounds (Fig. 6). The structures of misoprostol and carboprost are very similar and their elution and fragmentation are therefore almost identical, although the relative abundance of the major fragments is different. However, we obtained unique

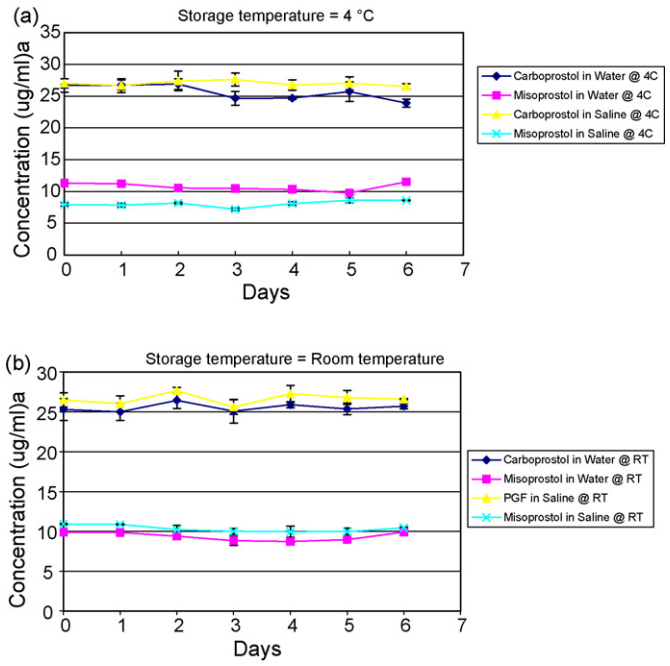

Fig. 7. Stability of misoprostol and carboprost dissolved in water for injection and saline at (a) 4 °C and (b) room temperature.

minor fragments in the two compounds in our MS analysis (Fig. 3). We selected the fragment at about 303.25 *m/z* for methyl carboprost and 267.19 *m/z* for misoprostol. The fragmentation of major ions is detailed in Table 1, but the selected ion fragmentation is unknown. Although detection limits were sacrificed somewhat by choosing minor fragments, they were still adequate and were as low as the μg/ml range, due to using a unique exact mass integration in Q-Tof to improve the signal-to-noise ratio.

Although atmospheric pressure chemical ionization (APCI) is claimed to be specific and sensitive for determination of misoprostol [7], we found that misoprostol was degraded in the high-temperature gas plasma. Williams et al. may have used the selected reaction-monitoring (SRM) mode of the LCMS which would have ignored the degraded products. Danielsson et al., in another study, used negative ion mode for misoprostol detection [11], but no unique fragment was generated to differentiate misoprostol from carboprost.

To verify whether low recovery of misoprostol from Cytotec tablet powder is due to its instability during extraction process or due to misoprostol entrapped in the cellulose paste from the excipient powder, we compared the recovery of misoprostol from water extract with that from placebo powder with the same extraction process and length of time. We also increased onefold of extraction time to verify if the stability of misoprostol was still maintained. We found there were significant differences in recovery of misoprostol from water extract and powder extract in the same extraction time but no significant difference in recovery with different extraction time but from the same matrix.

The recovery of misoprostol was about 100% from water extract but only about 47% from excipient powder. This indicates that misoprostol is stable during the extraction process because the level of misoprostol did not decrease during the sample processing in water. Misoprostol was stable even at longer extraction time because the recovery was about the same. Lower recovery from excipient powder indicates part of misoprostol should be entrapped or absorbed into cellulose powder that could not be released into water. A longer extraction time from the placebo powder did not produce a significant increase in concentration. It shows 5 min extraction time gives maximum extraction yield. Further extraction could not extract more. Also, since almost 100% misoprostol is recovered from water extract, this indicates low recovery from placebo powder is not due to low solubility of misoprostol but rather due to partition to the cellulose matrix. Also, we have tried to use ethanol extraction and reconstitute to 30% acetonitrile after evaporation. However, the yield was about 22%. It may be because the structure of cellulose collapses when contacting with organic solvent. Therefore, misoprostol is even more difficult to release.

Both carboprost and misoprostol were found to remain stable in solution water or saline when stored for up to 6 days at room temperature or at 4 °C. A slight drop in concentration of carboprost in water at 4 °C was found after 3 days. This may be because of susceptibility to the defrosting process during daily sampling. Unlike carboprost solution, misoprostol was not fully recoverable from water or saline solution and only about half of the amount contained in the Cytotec™ tablet appears to be retained in the excipient powder. This may explain why our results for umbilical vein injection of misoprostol fell short of those expected from preliminary published data using another prostaglandin E1 analogue [16]. Low recovery can be explained by the excipient formulation containing HPMC which is used to control-release hydrophobic drugs [17]. Since our ultimate aim is to derive a simple and safe extraction procedure for misoprostol for third world clinical use, higher efficiency methanol and ultrasonic extraction were not used in this study. These methods require complicated evaporation, aseptic reconstitution procedure, well-trained staff and expensive equipment which would not normally be available in a clinical setting. Although the recoveries are low, we can obtain a solution containing the required concentration of misoprostol by increasing the number of tablets for extraction, provided we know the percentage of recovery.

In short, we have developed and validated a method to simultaneously analyze carboprost, misoprostol and methyl carboprost for stability testing in a 10 min run. Our method could also further be adapted for biological fluid analysis by modifying the sample preparation steps. We have revealed that carboprost and misoprostol are stable in aqueous solution over a period of 6 days.

Acknowledgements

We gratefully thank the special equipment grant from the Faculty of Medicine, The Chinese University of Hong Kong for the Q-Tof Micro Mass Spectrometry.

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