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Quantitative HPLC analysis of mebeverine, mesalazine, sulphasalazine and dispersible aspirin stored in a Venalink monitored dosage system with co-prescribed medicines

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ABSTRACT

An HPLC method for the quantitative analysis of mebeverine HCl, 5-aminosalicylic acid (5-ASA), sulphasalazine and dispersible aspirin has been developed and then applied to these specific medicines when stored, with other medications, in Venalink blister packs (monitored dosage system) for periods of up to 35 days. Chromatographic separation was achieved on a reversed-phase C_{12} column with an isocratic mixture of methanol, water and acetic acid as the mobile phase. The method was validated regarding: accuracy, precision, detection limits, quantification limits, specificity and robustness.

1. Introduction

Monitored dosage systems (MDS) and compliance aids have been supplied to improve patient compliance with prescribed medicines for over 20 years for those patients who are having difficulties in managing their medications. MDS are supplied by pharmacies, sealed and labelled under a pharmacist's supervision, and with the provision of a medicines administration record chart, examples include: Manrex, heat foil-sealed; Venalink, cold-sealed; and Nomad, a recyclable cassette system that is tamper-sealed. Compliance aids are intended for use by a patient's carer and involve some elements of risk due to the containers being filled by a lay person, and a risk of accidental spillage. Examples available in recent years include Dosette and Medidos tablet boxes.

Typically, MDS and compliance aids comprise compartments or blister packs divided into the seven days of the week and four to six administration times each day for different dose times such as breakfast, lunch-, tea- and bed-time. Some advantages and disadvantages of these dosette boxes have been reported [1,2]. The advantages include helping to take care of patients on multiple medications (polypharmacy) at home so they do not need admission to hospital. MDS are helpful for patients who are on complex regimens and they are used to remind patients who often forget to take their medicines, so MDS can decrease dose and timing errors. The medicines are stored in an accessible way for patients with visual impairment, and allowing the administration of medicines by carers, but there is no reported quantitative analysis to-date obtained under pragmatic conditions, so how safe (quantitatively) are such MDS in practice for polypharmacy?

In this study we quantify four selected drugs in their medicines, dispensed in six different prescriptions, stored in combination with other medicines for a period of up to 5 weeks in Venalink blister packs, protected from heat and light. We report the first quantitative HPLC analysis of mebeverine, mesalazine, sulphasalazine and dispersible aspirin stored in a Venalink MDS with co-prescribed medicines.

2. Materials and methods

2.1. Chemicals and reagents

Mebeverine HCl was kindly supplied by Solvay Healthcare Ltd., UK. 5-ASA was purchased from Fluka, sulphasalazine and aspirin were purchased from Sigma. Methanol was of HPLC grade (Fischer Scientific). High purity water was prepared by use of a Millipore Milli Q system.

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2.2. Pharmaceutical dosage forms

Mebeverine tablets BP 135 mg (Generics Ltd., UK), cetrizine dihydrochloride 10 mg film coated tablets (TEVA UK Ltd.), simvastatin 40 mg film-coated tablets, lansoprazole 15 mg gastro-resistant capsules (TEVA UK Ltd.), paracetamol 500 mg tablets (Actavis, UK), bendroflumethiazide 2.5 mg tablets (Actavis, UK), aspirin 75 mg enteric coated tablets (Approved Prescription Services Ltd., UK), atenolol 50 mg tablets (TEVA UK Ltd.), Monomax SR 40 mg capsules (Trinity-Chiesi Pharmaceutical Ltd., UK), Fentazin (perphenazine) 2 mg tablets (Goldshield Pharmaceuticals Ltd., UK), co-codamol 30/500 mg tablets (Actavis, UK), Asacol (mesalazine) 400 mg MR tablets (Procter & Gamble Pharmaceuticals UK Ltd.), amitriptyline 25 mg tablets BP (TEVA UK Ltd.), buscopan 10 mg tablets (Boehringer Ingelheim Ltd., UK), Zamadol (tramadol) SR 100 mg capsules (MEDA Pharmaceuticals Ltd., UK), sulphasalazine 500 mg EC tablets (Haupt Pharma Berlin, Germany), aspirin 75 mg dispersible tablets (TEVA UK Ltd.), lisinopril 20 mg tablets (TEVA UK Ltd.), Cardozin XL 4 mg prolonged release tablets (Arrow Generics Ltd., Republic of Ireland) and Celebrax (celecoxib) 200 mg hard capsules (Pharmacia Ltd., UK).

2.3. Chromatography

The HPLC system consisted of a Jasco PU-980 pump coupled with a Jasco UV-15-75 detector and the output signals were recorded with a Servoscript SE-120 chart recorder. Compounds were separated on a reversed-phase analytical C_{12} column, Phenomenex, Synergi Max-RP 150 mm × 4.6 mm, 4 μm, at 20 °C at a flow rate of 1.5 ml/min. The isocratic mobile phases were: methanol, Milli Q water and acetic acid (40:59:1, v/v/v) for mebeverine HCl and 5-ASA, and (70:29:1, v/v/v) for sulphasalazine and aspirin. Each mobile phase was filtered through a nylon membrane filter (0.45 μm) and degassed by sonication prior to use. The injection volume was 20 μl and detection was at 263, 300, 365 and 270 nm for mebeverine HCl, 5-ASA, sulphasalazine and aspirin respectively. The detector sensitivity was 2.56 AUFS (as a whole tablet was used).

2.4. Statistical evaluations

A number of statistical methods were used including: mean ± SD, variance (SD^2), r^2, the Variance ratio test (*F*-test) and then the difference in means by Student's *t*-test at 95% confidence limits $p = 0.05$, as well as comparing our results against the official B.P. methods.

2.5. Preparation of the solutions

Standard (calibration) curves were prepared from diluted solutions, for each point $n = 6$. A stock solution of mebeverine HCl 2.7 mg/ml was prepared in mobile phase. Seven volumes (1–7 ml) of the stock solution were transferred into a series of 10 ml volumetric flasks, mixed with the internal standard (100 μl of 1% (w/v) 4-aminobenzoic acid in methanol) and then made up to 10 ml with the mobile phase. The solutions were filtered through a nylon membrane filter (0.45 μm) to obtain standard solutions of 0.27–1.89 mg/ml.

5-ASA, 10.0–90.0 mg (in 10.0 mg portions) was transferred into a series of 10 ml volumetric flasks. The analyte was dissolved in 0.1 M aqueous HCl (2–5 ml), mixed with the internal standard (500 μl of 1% (w/v) veratric acid in methanol) and then made up to 10 ml with 0.1 M aqueous HCl, filtered through a nylon membrane filter (0.45 μm) to obtain standard solutions of 1–9 mg/ml.

Sulphasalazine (500 mg) was dissolved in *N*,*N*-dimethylformamide (DMF, 2 ml) and the solution diluted to 100 ml with methanol. Seven different volumes (0.5–3.5 ml, in 0.5 ml steps) were transferred into a series of 10 ml volumetric flasks and mixed with the internal standard (1 ml of 0.1% (w/v) 4-*N*,*N*-dimethylaminobenzaldehyde) and then made up to 10 ml with methanol, filtered through a nylon membrane filter (0.45 μm) to obtain standard solutions of 0.25–1.75 mg/ml.

Aspirin, 10.0–70.0 mg (in 10.0 mg portions) was transferred into a series of 10 ml volumetric flasks. The analyte was dissolved in methanol (2–5 ml), mixed with the internal standard (800 μl of 1% (w/v) diclofenac sodium in methanol) and then the volume was made up to 10 ml with methanol, filtered through a nylon membrane filter (0.45 μm) to obtain standard solutions of 1–7 mg/ml.

On the day of analysis, individual tablets of the four selected drugs in six prescriptions were taken from the blister pack and dissolved with the chosen internal standard for quantitative analysis ($n = 6$). Each mebeverine tablet was transferred to a 100 ml volumetric flask, mixed with 50 ml of the mobile phase, sonicated (10 min), mixed with 1 ml of internal standard then made up to 100 ml with mobile phase, shaken well and 10 ml of the solution was filtered through a nylon membrane filter (0.45 μm) and the filtrate injected into the HPLC.

Each mesalazine tablet (for 5-ASA) was crushed in a glass mortar, transferred to a 100 ml volumetric flask and the mortar was washed with 0.1 M HCl (3 × 5 ml) and the washings were added to the volumetric flask. The flask was then sonicated (10 min), mixed with 5 ml of the internal standard then made up to 100 ml with 0.1 M HCl and shaken well and 10 ml of the solution was filtered through a nylon membrane filter (0.45 μm) and the filtrate was injected into the HPLC.

Each sulphasalazine tablet was crushed in a glass mortar, transferred to a 100 ml volumetric glass and dissolved in DMF (2 ml) and the mortar was washed with methanol (3 × 5 ml) and the washings were added to the volumetric flask and the volume was made up to 100 ml with methanol. The flask was then sonicated (10 min) and 10 ml of the solution was filtered through a nylon membrane filter (0.45 μm) then 1 ml of the filtrate was transferred to a 10 ml volumetric flask, mixed with 1 ml of the internal standard and made up to 10 ml with methanol and injected into the HPLC.

Each aspirin tablet was transferred to a 25 ml volumetric flask, dissolved in methanol (2–5 ml), mixed with 2 ml of the internal standard and made up to 25 ml with methanol. The flask was then sonicated (10 min) and 10 ml of the solution was filtered through a nylon membrane filter (0.45 μm) and the filtrate was injected into the HPLC.

In week 5, after 35 days of shaking 3 times daily, HPLC peaks of analytes (Fig. 1) from all four medicines studied were further analysed by high-resolution mass spectrometry (HR-MS) to compare with the isotope pattern data obtained from authentic samples.

2.6. Packing of the blister packs

The packing of each blister pack was performed with a cold seal process following the Venalink instructions which include 5 steps:

(1) A blister is placed through the holes into the bill bat of a pre-folded Venalink card.
(2) The Venalink is then loaded with the medications into the blisters.
(3) When all the required medication has been loaded, the release paper on the foil half of the card is removed.
(4) The foil half of the card is then folded onto the blister half of the card from the fold outwards, to ensure no air bubbles are trapped.
(5) The card is then briefly rolled to ensure that a good seal has been applied.

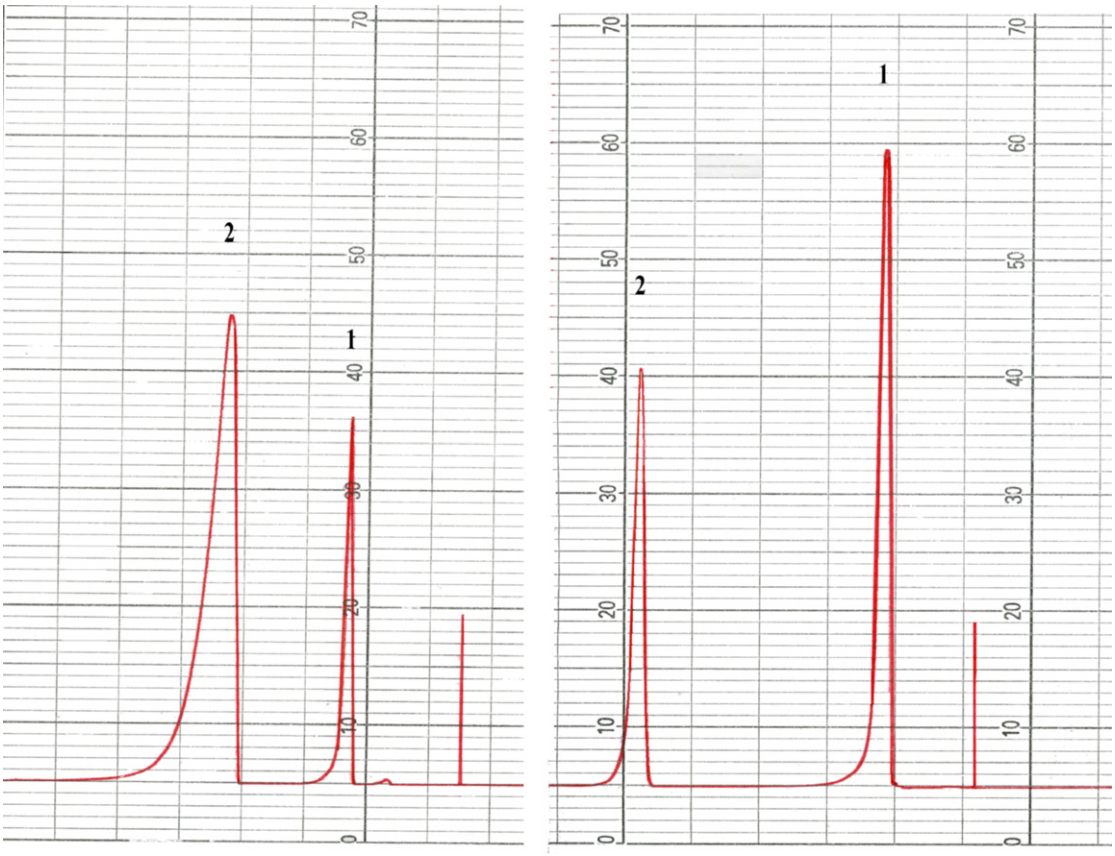

Fig. 1. Representative HPLC traces of (1) 4-aminobenzoic acid and (2) mebeverine HCl from mebeverine tablets (left) and (1) 5-ASA from Asacol MR tablets and (2) veratric acid (right).

2.7. Selection of the polypharmacy prescriptions

45 prescriptions from community pharmacies were collected and anonymised of any identifiable patient or prescriber data where mebeverine, 5-ASA (mesalazine, Asacol), sulphasalazine and dispersible aspirin were prescribed as solid dosage forms (e.g. tablets, capsules, slow-release products) alongside one or more other solid dosage form on the same prescription, and judged suitable for MDS dispensing. Our aim is to analyse quantitatively these four selected drugs in six selected (real) prescriptions in MDS:

R_x1. Mebeverine HCl 135 mg 84 tablets (one tablet three times daily).
Cetrizine 10 mg 28 tablets (one tablet daily).
Simvador 40 mg 28 tablets (one tablet at night).
Lansoprazole EC 15 mg 28 capsules (one capsule daily).
Paracetamol 500 mg 100 tablets (one to two tablets four times daily).
Bendroflumethiazide 2.5 mg 28 tablets (one tablet daily).

R_x2. Mebeverine HCl 135 mg 84 tablets (one tablet three times daily).
Aspirin EC 75 mg 28 tablets (one tablet daily).
Atenolol 50 mg 28 tablets (one tablet daily).
Monomax SR modified release 40 mg 28 capsules (one capsule daily).
Perphenazine 2 mg 28 tablets (one tablet daily).

R_x3. Mebeverine HCl 135 mg 100 tablets (one tablet three times daily).
Co-codamol 30 mg + 500 mg 100 tablets (one or two tablets up to four times daily).

R_x4. Mesalazine MR 400 mg 120 tablets (two tablets three times daily).
Mebeverine HCl 135 mg 84 tablets (one tablet three times daily).
Amitriptyline HCl 25 mg 28 tablets (one tablet at night).
Buscopan 10 mg 56 tablets (two tablets four times daily when required).
Tramadol MR 100 mg 60 capsules (one capsule four times daily).

R_x5. Sulphasalazine EC 500 mg 120 tablets (two tablets four times daily).
Aspirin 75 mg dispersible 28 tablets (one tablet daily).
Atorvastatin 40 mg 28 tablets (one tablet daily).
Bendroflumethiazide 2.5 mg 28 tablets (one tablet daily).
Doxazosin mr 4 mg 28 tablets (one tablet daily).
Lisinopril 20 mg 28 tablets (one tablet daily).

R_x6. Sulphasalazine EC 500 mg 120 tablets (2 tablets two times daily).
Celecoxib 200 mg 60 capsules (one capsule daily).

3. Results and discussion

3.1. What safety studies are reported for MDS?

The disadvantages of MDS, and particularly compliance aids, include errors caused by secondary dispensing or filling by carers, contamination with powder remaining from previously stored medicines in reused containers, and bacterial or fungal contamination. Also, they are only suitable for solid dosage forms that have to be swallowed, so buccal, sublingual, dispersible and effervescent dosages cannot be stored in these boxes as well as hygroscopic, photosensitive and cytotoxic medicines. Another important disadvantage is that long-term stability of medicines in these boxes has not been widely reported. They do not offer a child resistant closure. Solid dosage forms can move between compartments if a cassette box or blister pack is not closed properly or if it is dropped. The Medicines and Healthcare products Regulatory Agency (MHRA), UK, was made aware of a patient who took the incorrect dose of their medication because the individual compartments of the dosette boxes had not been sealed fully, allowing the tablets to migrate between individual compartments. No harm to the patient was reported, but in different circumstances and with different medication there is the potential for serious consequences to a patient's health [3]. Newer systems, e.g. Biodose and Venalink Wiegand, are now becoming available that overcome some of the current shortfalls of MDS systems, with claims of microbial resistance, child-safety, and the possibility of packing liquid or semi-solid formulations.

Table 1
Statistical analysis of the results from the analysis of mebeverine HCl ($n = 6$) and 5-ASA ($n = 6$) by the new HPLC method compared to the official B.P. methods [30].

Mebeverine HCl			Official B.P. method	5-ASA			Official B.P. method
Taken C (mg/ml)	Found C (mg/ml)	Recovery (%)		Taken C (mg/ml)	Found C (mg/ml)	Recovery (%)	
0.27	0.268	99.30		1	0.99	99.18	
0.54	0.541	100.32		2	1.99	99.88	
0.81	0.801	98.96		3	3.03	101.10	
1.08	1.09	101.09		4	3.99	99.87	
1.35	1.354	100.32		5	5.03	100.60	
1.62	1.61	99.47		6	5.91	98.63	
1.89	1.89	99.47		9	9.02	100.27	
Mean ± SD = 99.92 ± 0.73			99.83 ± 0.51	Mean ± SD = 99.93 ± 0.83			99.50 ± 0.58
$V = 0.53$			0.26	$V = 0.69$			0.33
$N = 7$			6	$N = 7$			6
$t = 0.25$			(2.20)[a]	$t = 1.06$			(2.20)[a]
$F = 2.03$			(4.39)[a]	$F = 2.11$			(4.39)[a]

[a] 2.20 is the theoretical t-value and 4.39 is the theoretical F-ratio at $p = 0.05$.

In his article in the *Pharmaceutical Journal* in 1992, Walker [4] collected information from about 53 pharmaceutical companies in the UK and each company was asked to indicate which of its solid dosage forms could not be transferred from its original pack and stored in a compliance device. The results obtained showed that although there are no short-term stability data available about these (compliance aid) boxes, Walker suggested that the majority of solid dosage forms could be transferred to compliance devices for a period of seven days with exceptions to this rule in some cases e.g. moisture and light sensitive tablets and unstable preparations, large solid dosage forms, medicines that deteriorate in contact with plastic and effervescent and dispersible tablets. Also, it was proposed that extensive stability studies be carried out to ensure that these compliance devices are safe to use. A leading article in the same (1992) *Journal* emphasized the need of short-term stability studies for all medicines especially in controlled dosage systems and the MHRA should have a policy and standards for the use of these secondary containers [5]. Correspondence to the *Journal* which followed this article supported the need to obtain short-term stability information and maintaining the highest professional standards for the use of compliance devices [6].

In an attempt to give a focus to the stability problems associated with these boxes, 50 pharmaceutical companies participated in a survey that covered 392 medicinal products, each product was given a stability code linked to the suitability for its use in dosette boxes. The results reported in 2006 that there is still a shortage of short-term stability data for the storage of medicines in dosette boxes [7]. Several companies suggested cutting around blister (packs) and putting the dosage form, still in the blister, into the dosette box, but this method should be avoided as there was a warning in the *Pharmaceutical Journal* which recorded two adverse cases, one of them fatal when a patient swallowed the tablet still in its blister resulting in intestinal perforation which required surgery, the patient developed a severe chest infection 48 h postoperatively and died [8].

Recently, the implications of storing paracetamol tablets in dosette boxes were reported [9]. The study included physicochemical stability tests e.g. physical characteristics of the tablets (weight, appearance, thickness, hardness, disintegration and dissolution rates) and these characteristics were evaluated according to the British Pharmacopoeia (B.P.) requirements while chemical stability was assayed by a reported text-book HPLC method that was used to quantify paracetamol in the presence of its degradation products and formulation excipients. The study was performed at times: zero (directly after sealing), one month and three months. The study proved that paracetamol tablets were stable in dosette boxes. Another study assayed the stability of furosemide tablets stored in dosette boxes that involved the investigation of physical, chemical and photo-stability over a period of eight weeks [10]. Results showed that furosemide tablets stored in these boxes developed a yellow discolouration starting from week 1 and although the colour change had no effects on the tablet content and physicochemical parameters of the tablet, as it is a surface effect, it is still an unacceptable change especially regarding patient acceptance and compliance and the patient's opinions about the quality of the dispensed medicine. Hence furosemide tablets stored into dosette boxes should be protected from light both in the pharmacy and at the patient's home by placing the dosette boxes in foil or cardboard.

3.2. Optimization of the LC procedure

HPLC analysis is commonly used for the quantitative determination of medicines and for stability studies. It has been used for the quantitative determination of mebeverine HCl [11–14] and its stability in tablets [15], for the determination of 5-aminosalicylic acid (5-ASA) [16–20] and the stability of 5-ASA and its metabolites in plasma [21,22], for sulphasalazine [23,24] and for aspirin [25–29].

The development of LC methods requires a suitable combination between the polarity of the analyte, the stationary and mobile phases to obtain good separation in a reasonable time. Two simple, practical and robust HPLC methods were developed, optimized and validated to quantify accurately the four chosen drugs. The methods were then applied to the four medicines stored in blister packs under the typical conditions of polypharmacy in MDS. An internal standard was used to improve inter-day accuracy in the measurements. These new HPLC conditions were developed after experiments with different ratios of organic solvents (typically, methanol, acetonitrile and tetrahydrofuran) in the mobile phase and finally an isocratic mobile phase of methanol:water:acetic acid (40:59:1, v/v/v) was selected for mebeverine HCl and 5-ASA and of (70:29:1, v/v/v) for sulphasalazine and aspirin. These two systems gave base-line resolution of each analyte from the internal standards. The mobile phases developed in this study can be easily used for both routine and quantitative quality control. They do not contain inorganic salt buffers which might block or damage the chromatographic column and equipment. Moreover, these mobile phases both result in short retention times, so small volumes of solvent are consumed with minor amounts of waste obtained. Retention times of 3.8, 1.3, 4.6 and 1.5 min were obtained for mebeverine HCl, 5-ASA, sulphasalazine and aspirin respectively and 1.8, 4.9, 2.0 and 7.2 min for their corresponding internal standards (Fig. 1), and results for the analysis of their standard solutions compared to the official B.P. methods [30] are given in Tables 1 and 2.

Table 2
Statistical analysis of the results from the analysis of sulphasalazine ($n = 6$) and aspirin ($n = 6$) by the new HPLC method compared to the official B.P. methods [30].

Sulphasalazine			Official B.P. method	Aspirin			Official B.P. method
Taken C (mg/ml)	Found C (mg/ml)	Recovery (%)		Taken C (mg/ml)	Found C (mg/ml)	Recovery (%)	
0.25	0.253	101.20		1	1.00	100.00	
0.50	0.508	101.62		2	1.96	98.04	
0.75	0.743	99.06		3	3.01	100.33	
1.00	0.990	99.03		4	4.03	100.75	
1.25	1.249	99.92		5	5.02	100.40	
1.50	1.498	99.86		6	6.01	100.16	
1.75	1.757	100.42		7	6.96	99.42	
Mean ± SD = 100.16 ± 0.99			99.94 ± 0.84	Mean ± SD = 99.87 ± 0.91			99.83 ± 1.05
$V = 0.98$			0.70	$V = 0.82$			1.10
$N = 7$			6	$N = 7$			6
$t = 0.42$			(2.20)[a]	$t = 0.07$			(2.20)[a]
$F = 1.40$			(4.39)[a]	$F = 1.34$			(4.39)[a]

[a] 2.20 is the theoretical t-value and 4.39 is the theoretical F-ratio at $p = 0.05$.

Table 3
Statistical analysis of the results from the determination of mebeverine HCl ($n = 6$) in R_x1 and R_x2 by the new HPLC method.

Day	Taken C (mg/ml)	R_x1				R_x2	
		Breakfast time		Bedtime		Found C (mg/ml)	Recovery (%)
		Found C (mg/ml)	Recovery (%)	Found C (mg/ml)	Recovery (%)		
0	1.35	1.35	100.43	1.34	99.92	1.35	100.12
1	1.35	1.34	99.92	1.34	99.71	1.35	100.53
7	1.35	1.35	100.53	1.36	100.94	1.34	99.51
14	1.35	1.36	101.14	1.34	99.30	1.35	100.32
21	1.35	1.35	100.12	1.36	100.94	1.36	101.14
28	1.35	1.34	99.51	1.36	101.14	1.36	100.73
35	1.35	1.36	101.14	1.33	99.10	1.35	100.53
Mean ± SD		100.34 ± 0.61		100.15 ± 0.84		100.41 ± 0.51	
V		0.36		0.72		0.26	
N		7		7		7	

3.3. Analysis of pharmaceutical formulations

Despite that it is not recommended to store aspirin dispersible tablets in such Venalink packs, we decided to store them along with five other medicines and then carry out a quantitative analysis of: mebeverine tablets in R_x1, 2, 3 and 4, mesalazine tablets in R_x4, sulphasalazine tablets in R_x5 and 6, and dispersible aspirin tablets in R_x5.

On each day of the analysis, brief and routine visual inspection of the tablet under analysis did not show any change in colour or signs of degradation of the tablet or the enteric coated film, especially in weeks 3, 4 and 5. Each week, quantitative HPLC analysis of the tablets showed a single peak for each analysed medicine and their internal standard with the same HPLC retention time and UV λ_{max} as the authentic and as the $t = 0$ sample. The ratio of peak areas was then calculated, and using the standard curve shown to be ~100% of each tablet's labelled amount (Tables 3–6).

Also, no additional peaks from the authentic medicine or from degradation on being stored in contact with other medicines in the same compartment were shown in the chromatogram. The quantitative assay of the medicines achieved indicated that there was no interference from the excipients commonly present in the other formulations. Furthermore, the results of the HR-MS (accurate within 5 ppm) showed the mass ion peaks $(M+H)^+$ of the analysed medicines without any obvious degradation and in the same isotopic ratio (data not shown): mebeverine $C_{25}H_{36}NO_5$ calc. 430.2588, found 430.2597 (authentic) 430.2574 (R_x); 5-ASA $C_7H_8NO_3$ calc. 154.0499, found 154.0498 (authentic) 154.0502 (R_x); sulphasalazine $C_{18}H_{15}N_4O_5S$ calc. 399.0758, found 399.0748 (authentic) 399.0770 (R_x); aspirin $C_9H_8NaO_4$ calc. 203.0315, found 203.0316 (authentic) 203.0320 (R_x). It was therefore concluded that, after 5 weeks in a Venalink pack, no degradation of mebeverine HCl, 5-ASA, sulphasalazine and aspirin formulated in their tablets was detected by this quantitative method.

Table 4
Statistical analysis of the results from the determination of mebeverine HCl ($n = 6$) in R_x3 and R_x4 by the new HPLC method.

Day	Taken C (mg/ml)	R_x3		R_x4	
		Found C (mg/ml)	Recovery (%)	Found C (mg/ml)	Recovery (%)
0	1.35	1.35	100.12	1.34	99.92
1	1.35	1.33	99.10	1.34	99.71
7	1.35	1.36	101.14	1.36	101.35
14	1.35	1.36	100.94	1.34	99.92
21	1.35	1.35	100.12	1.36	100.94
28	1.35	1.35	100.32	1.36	100.94
35	1.35	1.34	99.92	1.35	100.12
Mean ± SD		100.23 ± 0.67		100.41 ± 0.64	
V		0.45		0.41	
N		7		7	

Table 5
Statistical analysis of the results from the determination of 5-ASA ($n = 6$) in R_x4 by the new HPLC method.

Day	Taken C (mg/ml)	R_x4			
		Breakfast time		Bedtime	
		Found C (mg/ml)	Recovery (%)	Found C (mg/ml)	Recovery (%)
0	4	4.03	100.98	4.00	100.00
1	4	3.99	99.87	3.98	99.50
7	4	3.97	99.31	4.00	100.00
14	4	4.01	100.42	3.98	99.68
21	4	4.00	100.00	4.03	100.79
28	4	3.98	99.68	3.97	99.31
35	4	3.99	99.87	4.03	100.79
Mean ± SD		100.01 ± 0.53		100.04 ± 0.55	
V		0.29		0.21	
N		7		7	

3.4. Validation of the assay method

3.4.1. Linearity

The plots of authentic drug/internal standard peak area ratios versus the concentration were linear over the concentration range 0.27–1.89 mg/ml, 1–9 mg/ml, 0.25–1.75 mg/ml and 1–7 mg/ml for mebeverine HCl, 5-ASA, sulphasalazine and aspirin respectively. The calculated regression equations are: mebeverine HCl ratio = 3.6177 conc. + 0.52, 5-ASA ratio = 0.1352 conc. + 0.5499, sulphasalazine ratio = 5.0114 conc. + 0.1186 and aspirin ratio = 0.1793 conc. + 0.0808 where ratio is the authentic drug/internal standard peak area ratio and concentration is measured in mg/ml. A correlation coefficient (r^2) of 0.9998 was obtained for all four drugs under these analytical systems. The results of the analysis of the authentic drug showed excellent reproducibility in a statistical comparison (no significant difference at $p = 0.05$) to the results obtained using both the Variance ratio test (*F*-test) and then the Student's *t*-test i.e. the obtained results (Tables 1 and 2) were lower than Vogel's *F*- and *t*-test values.

3.4.2. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were determined at signal to noise ratios of 3:1 and 10:1 respectively. The LOD and LOQ were found to be: 0.05 and 0.5 ng/ml for mebeverine HCl, 0.4 and 1 ng/ml for 5-ASA, 0.1 and 1 ng/ml for sulphasalazine, and 50 and 300 ng/ml for aspirin, proving that the method is sensitive.

3.4.3. Specificity

No interference from the diluents, other excipients or any impurities were observed, indicating a high degree of specificity for these methods of quantification of drug in mebeverine HCl, mesalazine, sulphasalazine and aspirin tablets.

3.4.4. Precision and accuracy

The intra-day precision of the method was determined by assaying 6 injections of the same concentration for mebeverine HCl, 5-ASA, sulphasalazine and aspirin within the same day and this was expressed as the relative standard deviation (RSD, %) which was: 0.49, 0.59, 0.38 and 0.48% for mebeverine HCl, 5-ASA, sulphasalazine and aspirin respectively. The inter-day precision was determined by assaying a sample of the same concentration of mebeverine HCl, 5-ASA, sulphasalazine and aspirin on three successive days and was expressed as RSD (%) which was: 0.62, 0.66, 0.45 and 0.36% for mebeverine HCl, 5-ASA, sulphasalazine and aspirin respectively indicating the high precision of the methods. The accuracy of these new methods was determined by assays of authentic drug. In individual tablets, the amount of mebeverine HCl assayed in mebeverine HCl tablets in four prescriptions ranged from 100.23 to 100.41%, 5-ASA from mesalazine tablets ranged from 100.01 to 100.04%, sulphasalazine assayed in two prescriptions ranged from 100.62 to 100.68% and aspirin ranged from 100.97% ($n = 6$) which confirmed the accuracy of the method. These individual tablets were therefore all shown to lie within the B.P. requirements that the concentration of these medicines in their tablets is not less than 95% and not more than 105% of the labelled concentration.

3.4.5. Robustness

In order to evaluate the robustness of the method, small changes in the chromatographic conditions were made, such as the percentage of methanol (35 and 45% for mebeverine HCl and 5-ASA and 65 and 75% for sulphasalazine and aspirin) and flow rate (1.4 and 1.6 ml/min). The obtained results showed a small change in the retention time, but there are no important effects on the analysis, confirming the robustness of the proposed method. Also, not unexpectedly, changing between HPLC machines and involving different operators in repeats of the same sample did not affect the quantitative analysis. Thus, the assay is robust, reproducible,

Table 6
Statistical analysis of the results from the determination of sulphasalazine ($n = 6$) in R_x5 and 6 and aspirin ($n = 6$) in R_x5 by the new HPLC method.

Day	Taken C (mg/ml)	Sulphasalazine				Aspirin R_x5		
		R_x5		R_x6		Taken C (mg/ml)	Found C (mg/ml)	Recovery (%)
		Found C (mg/ml)	Recovery (%)	Found C (mg/ml)	Recovery (%)			
0	0.5	0.503	100.62	0.502	100.5	3	3.04	101.54
1	0.5	0.507	101.42	0.504	100.9	3	3.03	101.17
7	0.5	0.505	101.02	0.503	100.6	3	3.02	100.79
14	0.5	0.501	100.22	0.505	101.0	3	3.04	101.54
21	0.5	0.497	99.42	0.506	101.3	3	3.01	100.42
28	0.5	0.507	101.42	0.503	100.6	3	3.04	101.35
35	0.5	0.501	100.2	0.499	99.9	3	3.00	100.00
Mean ± SD		100.62 ± 0.73		100.68 ± 0.44		100.97 ± 0.59		
V		0.53		0.19		0.35		
N		7		7		7		

practical and specific for the authentic drugs being assayed. It is quantitative within the parameters of HPLC retention time, UV λ_{max} and HR-MS including the same isotopic ratio pattern, all data matching both the authentic and the $t = 0$ samples. As these quantitative assays followed from those data with no significant variation, we did not carry out a forced-degradation study introducing heat, light or extremes of pH, oxidants, radicals or enzymes. We quantified the four drugs from their medicines against both their authentic and $t = 0$ samples while keeping the storage conditions close to those used by patients for our chosen six prescriptions.

4. Conclusions

The two new HPLC assay methods developed for mebeverine HCl, 5-ASA, sulphasalazine and aspirin are precise and rapid. The statistical analysis shows that the methods are both reproducible and selective for the analysis of mebeverine HCl, 5-ASA, sulphasalazine and aspirin as authentic drugs and in their pharmaceutical (solid dosage) formulations. The isocratic mobile phases, affording retention times of less than 10 min, were found to have practical advantages for the ease of use of these methods. No degradation of these four drugs in tablets was detected in these quantitative HPLC methods. We conclude that these tablets, stored in Venalink blister packs, remain stable for at least five weeks (e.g. one week of advanced packing and 4 weeks supply). However, the normal handling i.e. many times daily over many days during the latter part of the storage time led to rupture of a few of the blister seals so the remaining tablets were exposed to increased levels of air and humidity; therefore, the attention of the patient should be drawn to the storage of these blister packs away from light, heat and (where possible) humidity. This is the first quantitative study of polypharmacy in Venalink blister packs. The analytes remained stable for up to five weeks, even dispersible aspirin upon which there is something of a moratorium in such blister packs. At least for these four medicines in these six prescriptions, we have demonstrated experimentally that, under typical MDS conditions, the tablets remain intact with no deviation from the nominal amount of drug in each formulation.

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