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Determination of indinavir, potassium sorbate, methylparaben, and propylparaben in aqueous pediatric suspensions

Dawn M. Kreuz *, Angela L. Howard, Dominic Ip

Department of Pharmaceutical Analysis and Control, Merck Research Laboratories, West Point, PA 19486, USA

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Abstract

A gradient, reversed phase, HPLC method was developed for simultaneous analysis of potassium sorbate, methylparaben, propylparaben, and indinavir in aqueous suspensions that contain a proprietary orange flavoring and Magnasweet® sweetener enhancer (MacSanrews and Forbes Company, Magnasweet® product brochure). The chromatographic separation is performed on an Eclipse XDB-C8 column using a gradient run with an analysis time of 35 min. The mobile phase consists of acetonitrile and acetonitrile:citrate buffer, pH 4.0 (20:80 v/v). The method successfully separates the three preservatives, indinavir (active ingredient), the orange flavoring, the Magnasweet® species, and the indinavir lactone degradate. Recovery, linearity, and precision results for the three preservatives and indinavir are described. The method applies to two types of formulations: Xanthan Gum suspension and NanoSystems suspension.

Keywords: CRIXIVAN®; Indinavir; Pediatric suspensions; Potassium sorbate; Methylparaben; Propylparaben; Reversed phase HPLC

1. Introduction

Indinavir (Fig. 1) has been found to be a potent and specific in vitro inhibitor of the human immunodeficiency virus Type 1 (HIV-1) encoded protease [2–5]. HIV has been identified as the causative agent of acquired immune deficiency syndrome (AIDS) [2,3,6]. Indinavir has been approved for marketing in the USA, Canada, Australia and many countries in Europe, Asia, and

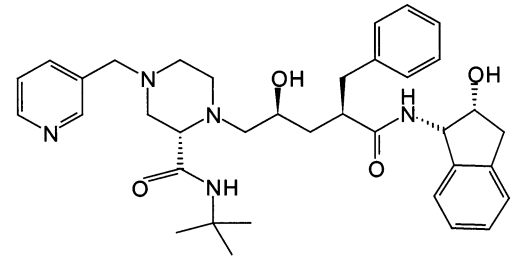

Fig. 1. Indinavir.

* Corresponding author. Tel.: +1-215-6528478.

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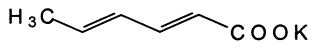

Fig. 2. Potassium sorbate.

South America under the trade name CRIXIVAN® for the treatment of HIV infection. The adult formulation is formulated in a hard gelatin capsule. Studies have been on-going for formulating indinavir in a pediatric suspension since not all children infected (<4 years old) are able to swallow capsules. Two aqueous pediatric formulations were developed for human clinical study: Xanthan Gum suspension and NanoSystems suspension.

The preservation of the drug suspension is extremely important, especially since this drug is used for immuno-compromised patients and is packaged in a multiple dose container. Potassium sorbate (Fig. 2), methylparaben (Fig. 3) and propylparaben (Fig. 4), which are used routinely for antimicrobial preservation, are used as preservatives. Methylparaben and propylparaben are used together since they have a synergistic effect [7]. Monitoring of the parabens is critical since they have been shown to absorb to plastic containers [7] which are preferred for pediatric products. Therefore, monitoring of the preservatives on a real time basis with the drug during product stability was a high priority in methods development.

An assay method for determination of indinavir, its lactone degradate (Fig. 5), potassium sorbate, methylparaben, and propylparaben was needed to support clinical and stability studies. The hydrolytic cleavage of the amide bond of indinavir under heat, acidic, or basic conditions is the primary mode of degradation which induces the formation of lactone and *cis*-aminoindanol (Fig. 6). The *cis*-aminoindanol is produced in an equimolar amount to the lactone, so it was not

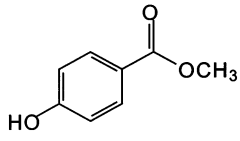

Fig. 3. Methylparaben.

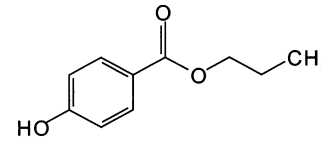

Fig. 4. Propylparaben.

necessary to monitor its formation. The goal was to have one method to assay the active, the lactone degradate, and the preservatives simultaneously. Various isocratic, reversed phase high-performance liquid chromatographic (HPLC) methods have been published for the determination of indinavir. Columns that have been used to resolve indinavir include a waters symmetry C8 [2], a Keystone BDS Hypersil C8 [4], and a Zorbax RX-C8 [6]. Mobile phases consisted of acetonitrile/water mixtures modified with various acids and buffers. Numerous methods are available in literature for the determination of preservatives, including sorbic acid (as opposed to potassium sorbate), methylparaben, and propylparaben. Some of the ones reviewed were isocratic, reversed phase HPLC methods using Waters μBondapak C18 [8,9], Perkin–Elmer C18 [10], and an S5 ODS 2 [11]. The mobile phase consisted of acetonitrile or methanol/water mixtures, modified with various acids and buffers. An ion pair reagent, trimethylammonium bromide, in an acetonitrile/phosphate buffer mobile phase was used with a nucleosil 5C18 column to resolve highly polar food additives from weakly polar ones [12].

The indinavir pediatric suspensions contain several excipients which absorb at the wavelength of interest (260 nm) for the drug and degradate in these formulations. A proprietary orange flavoring, povidone (PVP) (Fig. 7), and hydroxypropyl

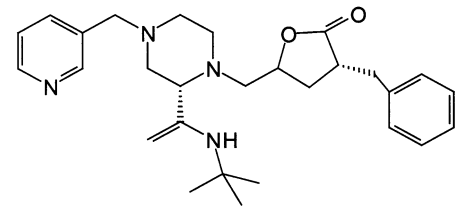

Fig. 5. Lactone degradate.

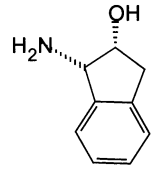

Fig. 6. *Cis*-aminoindanol degradate.

cellulose absorb in the desired UV range at low levels in the formulations. Magnasweet® (Fig. 8), developed by MacAndrews and Forbes Company to mask bitter aftertastes, to enhance natural and artificial flavors, and to improve sweetener systems [1], contains numerous peaks in these formulations. The gradient HPLC method that was developed successfully separates the three preservatives, indinavir, the orange flavoring, the Magnasweet® species, and the lactone degradate simultaneously.

2. Materials

2.1. Equipment

A Spectra system AS3000 HPLC consisting of autosampler, pump, and variable wavelength detector was used. The Eclipse XDB-C8 column, supplied by MAC-MOD, was 3.0 × 150 mm and 5 μm particle size. The Wrist Action Shaker was a model 75 from Burrell. The stir plates used were a Variomag unit from Electronicruhrer Multipoint HP and a Lab-Line Multi-Magnestir from Lab-Line Instruments, Inc. The centrifuge used was an Eppendorf model 5414.

2.2. Reagents

Acetonitrile was Fisher Optima grade. Citrate buffer was prepared using citric acid monohydrate, certified ACS, and sodium citrate dihydrate, certified. The suspensions were prepared to contain the analytes mentioned above in-house. Indinavir (monohydrate, free base) was obtained from the Chemical Data Department of Merck, Rahway, NJ. Water was Milli-Q purified from an in-house source.

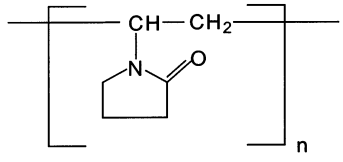

Fig. 7. Povidone.

3. Methods

3.1. Standard preparation

Standard solutions were prepared by performing serial dilutions of individual solutions of each preservative and then spiking the appropriate volumes of each preservative standard solution into a stock solution of the indinavir standard so that the analytes were all present at their respective method concentrations (2.7 μg potassium sorbate ml^{-1}, 0.6 μg methylparaben ml^{-1}, 0.06 μg propylparaben ml^{-1}, and 0.2 mg indinavir ml^{-1}). The sample preparation diluent consisted of acetonitrile:0.02 M citrate buffer, pH 4.0 (20:80% v/v). The methylparaben, propylparaben, and active were first dissolved in approximately 10 ml of acetonitrile before diluting to volume with diluent. Dissolution in acetonitrile was needed due to low aqueous solubility of the drug and parabens.

3.2. Sample preparation

Suspensions were shaken on a wrist action shaker for 30 min. Samples were taken by weight for analysis using a glass syringe and cannula since they were generally foamy after shaking. From the syringe, the sample was introduced directly into the flask for dissolution and analyte extraction. Specific gravity was measured using a 10-ml pycnometer so that suspension sample weights could be converted to volume. For each composite assay sample, 1 ml of suspension from each of three bottles was pooled together in a 1000 ml flask. For each dosage uniformity sample, 1 ml of suspension was transferred to a 500 ml flask. Flasks were filled to 80% volume with diluent (20% acetonitrile:80% 0.02 M citrate buffer, pH 4.0) and stirred 15 min. The acetonitrile level

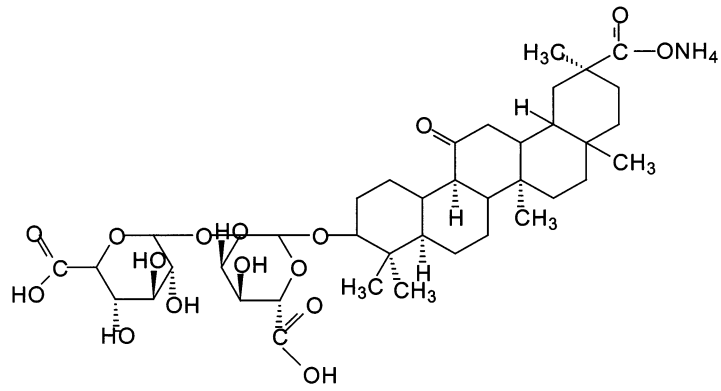

Fig. 8. Monoammonium glycyrrhizinate, primary flavoring constituent of the Magnasweet® product line.

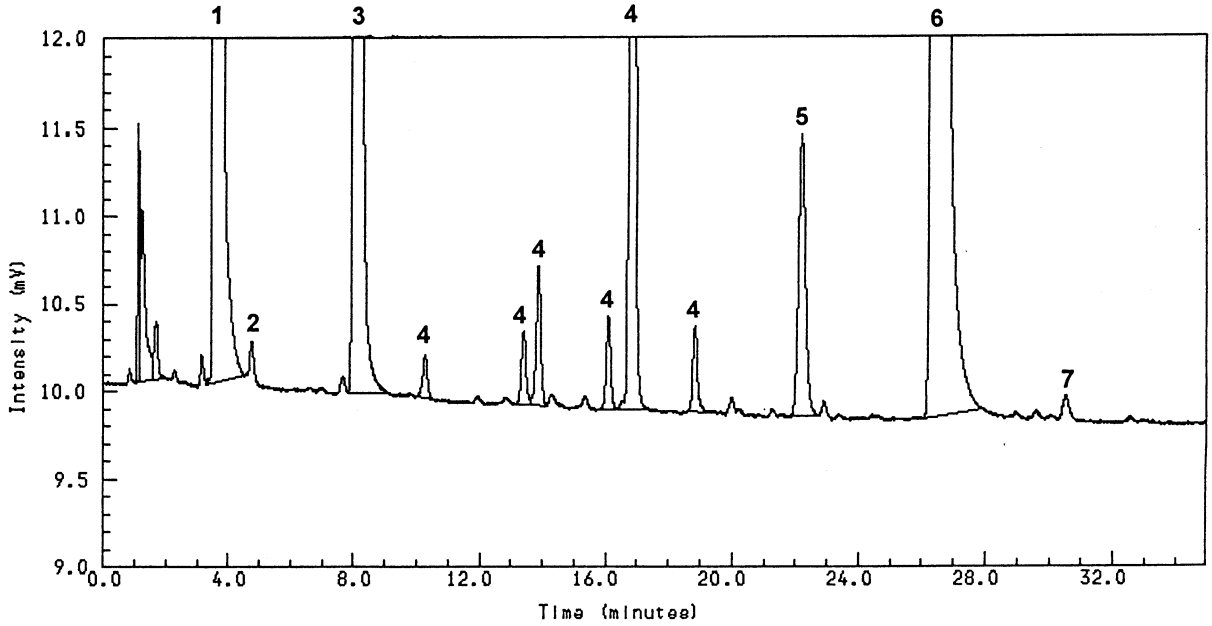

Fig. 9. Chromatogram of NanoSystem suspension: (1) potassium sorbate, (2) orange flavoring, (3) methylparaben, (4) Magnasweet®, (5) propylparaben, (6) indinavir, (7) lactone degradate.

was limited to 20% due to the gradient conditions required to resolve the early-eluting peaks. Since the organic level in the diluent was limited, the pH of the aqueous portion of the diluent was limited to 4.0 for solubility reasons. The solubility of the drug decreases rapidly between pH 4.0 and 5.0. Citrate buffer at pH 4.0 mixed with 20% acetonitrile offered adequate solubility of the drug. After diluting to volume, samples were further diluted 9–25 ml for composite assays, and 5–10 ml for dosage uniformity. Samples were centrifuged prior to analysis.

3.3. Recovery solutions

Since the formulations for the Xanthan Gum and NanoSystems suspensions were similar, the method was validated simultaneously by combining the excipients of both formulations. The recovery experiments were performed in two

separate studies: validation of the preservatives and validation of the active.

In the first study, a solution was prepared by adding the appropriate levels of excipients (from both the Xanthan Gum and NanoSystems suspensions) and active to diluent (20% acetonitrile: 80%

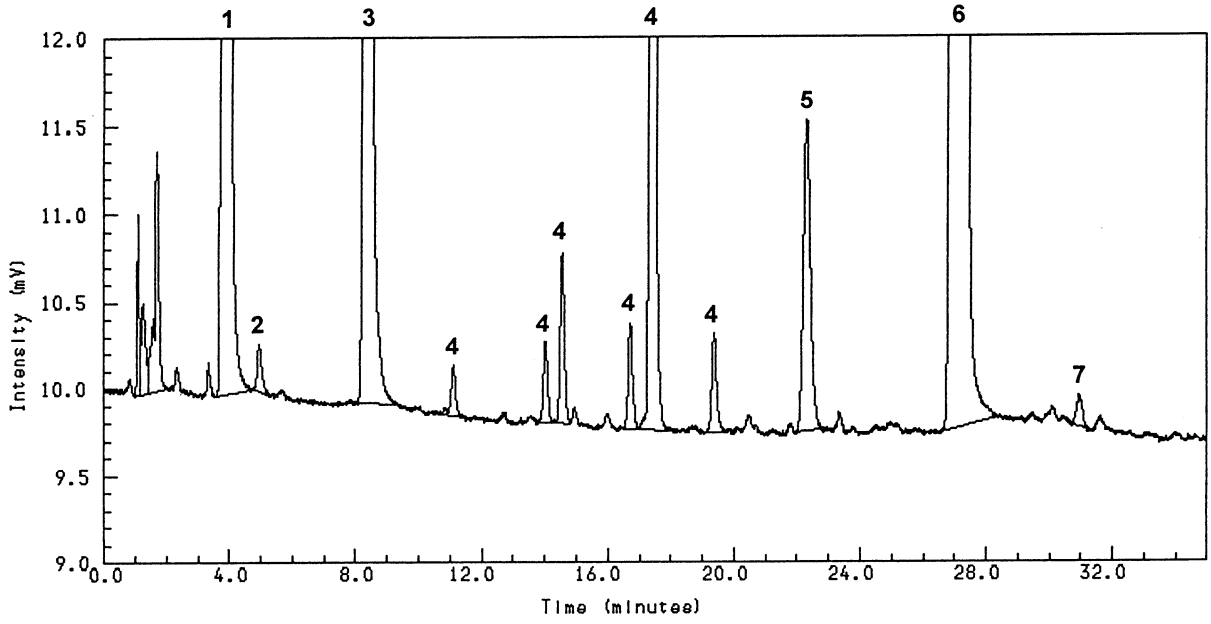

Fig. 10. Chromatogram of Xanthan Gum suspension: (1) potassium sorbate, (2) orange flavoring, (3) methylparaben, (4) Magnasweet®, (5) propylparaben, (6) indinavir, (7) lactone degradate.

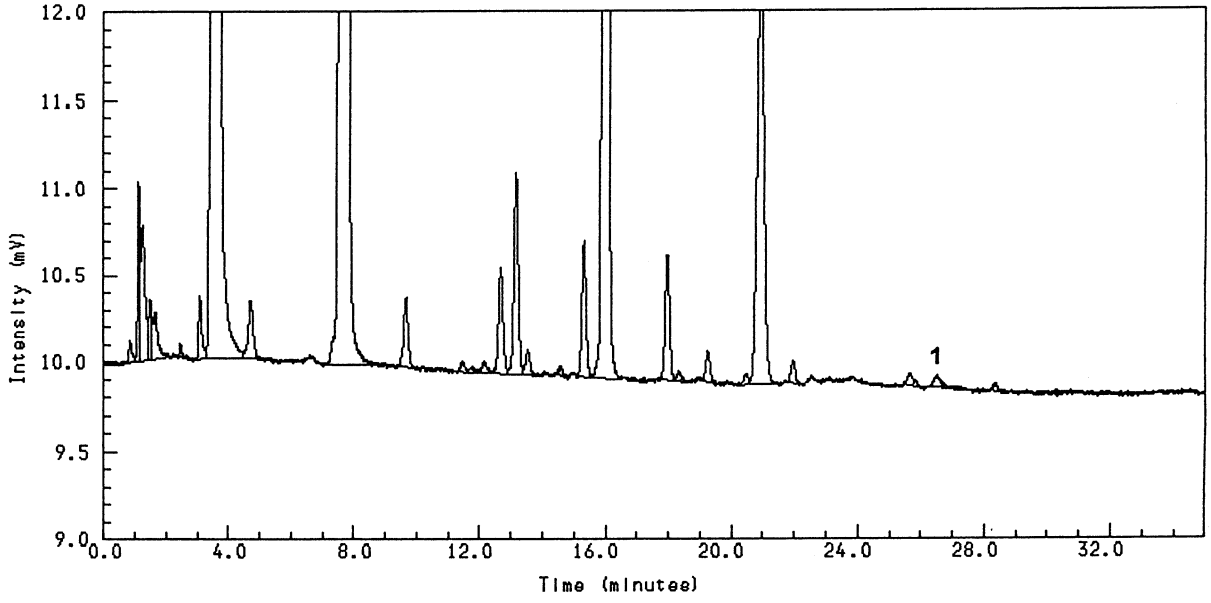

Fig. 11. Chromatogram of combined suspension vehicle for Xanthan Gum and NanoSystem suspensions to demonstrate excipient interference of indinavir analysis: (1) retention time of indinavir.

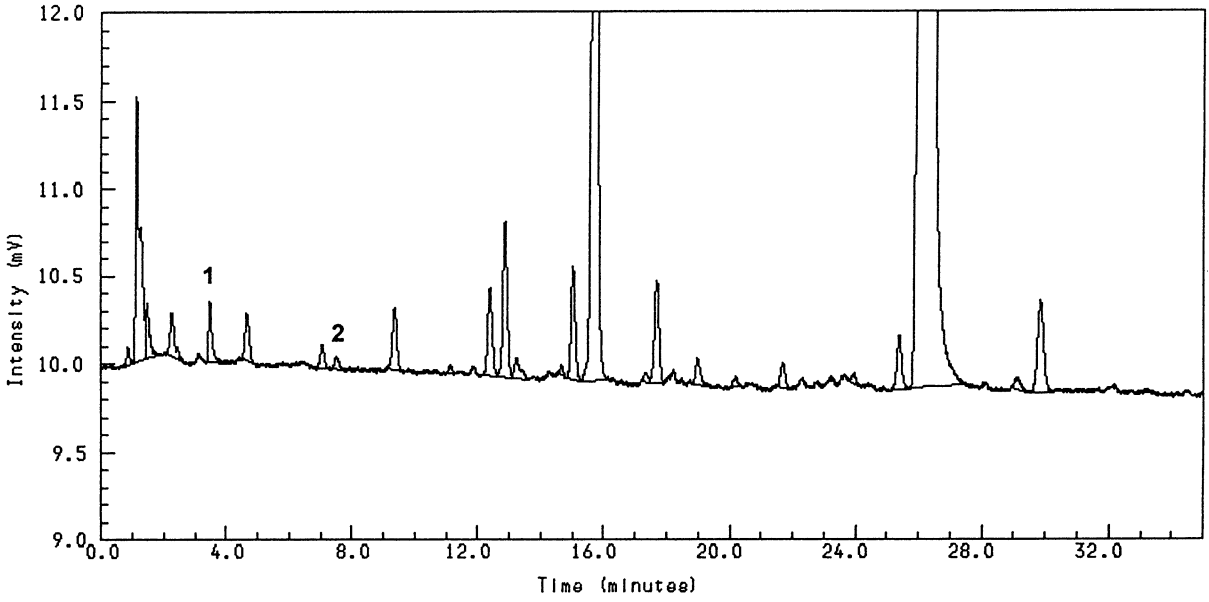

Fig. 12. Chromatogram of combined suspensions without the preservatives to demonstrate excipient interference of potassium sorbate and methylparaben analyses: (1) retention time of potassium sorbate, (2) retention time of methylparaben.

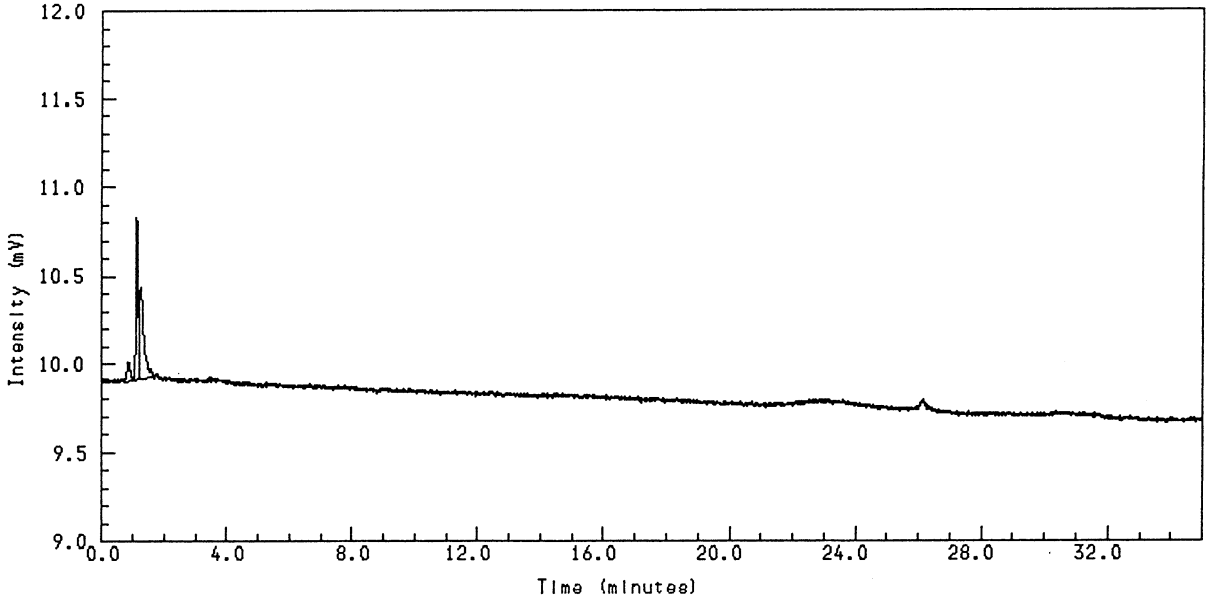

Fig. 13. Chromatogram of the diluent.

0.02 M citrate buffer, pH 4.0, v/v) so that their concentrations represented 100% levels in solution. Preservative recovery solutions were prepared in duplicate by spiking small volumes of a stock solution containing the three preservatives into the diluent solution containing excipients and active. The preservative levels of this final solution ranged from 55 to 161% of the method concentrations (2.7 μg potassium sorbate ml^{-1}, 0.6 μg methylparaben ml^{-1}, 0.06 μg propylparaben

Table 1
Recovery studies of preservatives and indinavir in pediatric suspension

Spike level of potassium sorbate	% Recovery of potassium sorbate	Spike level of methylparaben	% Recovery of methylparaben	Spike level of propylparaben	% Recovery of propylparaben	Spike level of indinavir	% Recovery of indinavir
53.9% A	100.8	54.0% A	99.3	54.6% A	101.3	46% A	99.6
53.9% B	100.1	54.0% B	98.7	54.6% B	100.8	49% B	99.4
80.9% A	100.4	80.0% A	100.5	81.9% A	102.3	74% A	99.2
80.9% B	100.1	80.0% B	100.1	81.9% B	100.8	71% B	98.7
107.8% A	99.0	106.6% A	99.6	109.2% A	101.3	96% A	98.8
107.8% B	99.3	106.6% B	99.9	109.2% B	100.9	96% B	98.7
134.8% A	99.1	133.3% A	99.9	136.5% A	101.0	122% A	98.1
134.8% B	98.9	133.3% B	99.7	136.5% B	101.1	125% B	102.2
161.7% A	98.4	160.0% A	99.6	163.9% A	101.3	146% A	98.3
161.7% B	98.5	160.0% B	99.7	163.9% B	100.8	141% B	98.6
Mean	99.5		99.7		101.2		99.2
RSD	0.8%		0.5%		0.4%		1.2%

ml^{-1}). Two solutions containing 0% preservatives were also prepared.

In the second study, the recovery of indinavir was assessed by analyzing solutions composed of suspension vehicle (placebo) at 100% method concentration spiked with known amounts of bulk drug at levels ranging from 46 to 146% of the method concentration (0.2 mg indinavir ml^{-1}). Active recovery solutions were prepared in duplicate. Two solutions containing 0% indinavir were also prepared.

Both sets of recovery solutions were stirred for 15 min and then centrifuged before analysis.

3.4. Quantitation by HPLC

Sample analyses and recovery studies used the same chromatographic parameters. The mobile phase consisted of (A) 20% acetonitrile: 80% 0.02 M citrate buffer pH 5.0 (v/v) and (B) acetonitrile. The buffer was premixed to extend its expiration and to improve mixing during the gradient. The following linear gradient was used: 0 min, 100% A; 35 min, 70% A and 30% B; 36 min, 100% A; 46 min, 100% A. Flow rate was 0.5 ml min^{-1}. The ultraviolet absorbance (UV) detector was set at 260 nm. Injection volume was 50 μl. Run time was 35 min for analysis and 11 min to re-equilibrate.

4. Results and discussion

The chromatographic conditions were developed for the Xanthan gum formulation. The goal during development was to have one method

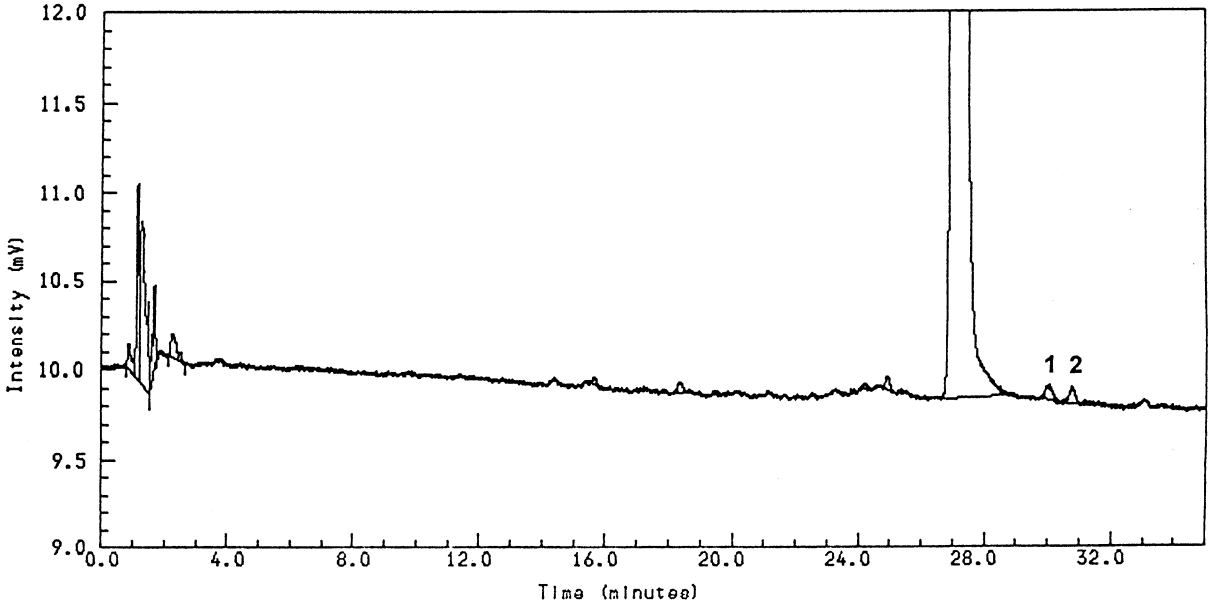

Fig. 14. Chromatogram of bulk indinavir: (1) bulk impurity, (2) lactone degradate.

Table 2
Linearity of preservatives and indinavir in pediatric suspension

Analyte	Linear range	Intercept	Slope	R^2
Potassium Sorbate	1.439 4.318 μg ml^{-1}	18607	883 800	1.000
Methylparaben	0.3348–0.9917 μg ml^{-1}	70.055	560 130	1.000
Propylparaben	0.0339 0.1016 μg ml^{-1}	24.559	485 180	1.000
Indinavir	0.1002 0.3144 mg ml^{-1}	7626.9	33 437 000	0.999

Table 3
Injector precision-reproducibility of area counts

Analyte	Average area counts (n = 10)	RSD (%)
Potassium sorbate	2476 858	1.1
Methylparaben	361 835	1.4
Propylparaben	31 622	0.8
Indinavir	6 533 270	1.0

Table 4
Method precision of preservatives and indinavir in pediatric suspension

Analyte	RSD (%)
Xanthan gum	
Potassium sorbate	2.6
Methylparaben	2.9
Propylparaben	2.7
Indinavir	2.7
NanoSystems	
Potassium sorbate	0.8
Methylparaben	0.7
Propylparaben	0.8
Indinavir	0.8

which could be used for determination of the active as well as the lactone degradate and the preservatives.

Initial development began by collecting UV spectra on all of the components to determine which ones would absorb at the maximum absorbance of indinavir and its lactone degradate, 260 nm. A sample of diluted suspension was injected using isocratic method conditions developed for a previous indinavir suspension formulation which did not contain Magnasweet®. This method used a Keystone BDS Hypersil C8 column, 4.6 × 150 mm and a mobile phase consisting of 37% acetonitrile: 63% 0.02 M citrate buffer, pH 5.0. The excipients which absorb at 260 nm and at the method concentration of a prepared assay solution are Magnasweet®, the orange flavoring, povidone (PVP), and hydroxypropyl cellulose. The Magnasweet® species contained many peaks which co-eluted with the preservatives and the active in the isocratic run at the wavelength of interest of this formulation, 260 nm. From these preliminary studies, it was evident that a gradient method would be required to resolve all of the excipients, the lactone degradate, and the indinavir bulk impurities from the four analytes.

Various gradient conditions were evaluated. The BDS Hypersil C8, 4.6 × 150 mm column that was used for a previous indinavir suspension and a MAC-MOD Eclipse XDB C8, 3.0 × 150 mm, 5 μm were evaluated. Preliminary gradients that were evaluated on these columns showed that the Eclipse XDB C8 column gave better resolution, so it was selected for further development of the gradient conditions. The small bore of this column (3 mm) was beneficial. It enhanced the sensitivity due to peak compression in the UV cell and saved on mobile phase since the flow rate would be reduced by more than 50% compared to a 4.6 mm i.d. column.

The mobile phase components selected for development were 0.02 M citrate buffer pH 5.0: acetonitrile. The citrate buffer in the mobile phase was adjusted to pH 5.0 to yield good indinavir peak shape since the pK_a of the drug is 3.7. The two solvents were mixed 80% 0.02 M citrate pH 5.0: 20% acetonitrile in component A to prolong the expiration of the citrate buffer as well as to help in

Table 5
Reproducibility of retention times

Analyte	Retention time (min, n = 10)	RSD (%)
Xanthan gum		
Potassium sorbate	3.48	0.2
Methylparaben	7.55	0.3
Propylparaben	20.72	0.2
Indinavir	26.18	0.2
Lactone Degradate	29.88	0.2
NanoSystems		
Potassium sorbate	3.43	0.3
Methylparaben	7.63	0.1
Propylparaben	21.35	0.1
Indinavir	25.81	0.1
Lactone degradate	29.80	0.1

the mixing during the gradient. Component B was 100% acetonitrile. Various gradient conditions were evaluated using these two components. The following linear gradient conditions gave the best resolution of the components in the least amount of time: 0 min, 100% A; 35 min, 70% A and 30% B. Increasing the time of the gradient, thereby decreasing the acetonitrile rate, causes the Magnasweet® to be retained slightly longer with respect to propylparaben. Decreasing the time of the gradient, thereby increasing the rate of acetonitrile, causes the Magnasweet® to elute slightly faster with respect to propylparaben. This observation is important to note since several small peaks from Magnasweet® elute near the propylparaben peak. If the indinavir and/or its lactone degradate peak do not elute in the analysis time of 35 min, a holding time must be added to the end of the gradient rather than changing the gradient rate. When tested on an HP model 1090 HPLC, it was determined that the mobile phase had to be held constant at 100% A for 2 min before the gradient was started since the void volume on this instrument was smaller than the Spectra System model AS3000.

By using a gradient method, the three preservatives were resolved from the excipients with only minor interferences, and the indinavir and preservative assays were achieved via one method. A chromatogram of a sample of the NanoSystems suspension is shown in Fig. 9. The *cis*-aminoindanol degradate elutes in the solvent front. Potassium sorbate elutes as a peak at a retention time of 3.6 min, the orange flavoring at 4.8 min, methylparaben at 8.1 min, propylparaben at 22.2 min, indinavir at 26.5 min, and lactone at 30.6 min. Magnasweet® elutes as a series of peaks starting at 7–23 min, with the largest peak at 16.8 min. A chromatogram of the Xanthan Gum, as shown in Fig. 10, is very similar. During validation, the interferences were quantitated. The povidone and hydroxypropyl cellulose co-eluted with the potassium sorbate, and some peaks from the Magnasweet® co-eluted with methylparaben and indinavir, but the interferences were insignificant (0.1% claim potassium sorbate, 0.2% claim methylparaben, $< 0.1\%$ claim indinavir). The minor interferences from excipients are demonstrated in Figs. 11 and 12. Chromatograms of the diluent and the bulk drug are shown in Figs. 13 and 14 for reference. The validation applies to both formulations since the excipients of these two formulations were combined for the testing.

Table 1 shows recovery studies for the preservatives and the active. Individual recovery values varied from 98 to 102% for each analyte at each spiked level. The average recovery across each analyte was 99–101%.

The theoretical concentrations of the recovery studies were plotted against the area counts for each respective peak. Table 2 shows the linearity range, intercept, and slope of the four analytes. The calibration graphs were constructed from two sample preparations each of five concentrations. The least squares regression fit showed excellent linearity ($R^2 \geq 0.999$) in the defined ranges for all analytes.

Injection precision was assessed by making ten replicate injections of a standard solution which contained the four analytes at their method concentrations. Table 3 shows injector reproducibility since the relative standard deviations were $< 2\%$ for each component.

Method precision was determined successfully by analyzing ten aliquots of sample suspension from both formulations. Table 4 shows that the relative standard deviations of the results in mg ml^{-1} suspension are less than 3% for each analyte. Table 5 shows the reproducibility of the retention times. The relative standard deviations were less than 0.4% for the four analytes.

The limit of detection (LOD) for indinavir, as measured by a signal-to-noise ratio of 2 or 3:1, was determined to be 10 ng ml^{-1}, or 0.005% of the standard concentration. The limit of quantitation (LOQ) for indinavir, as measured by a minimum signal-to-noise ratio of ten, was determined to be 60 ng ml^{-1} solution, or 0.03% of the standard concentration. Estimated LOD's for potassium sorbate, methylparaben, and propylparaben are 0.3, 0.8, and 1 ng ml^{-1}, respectively. Estimated LOQ's are 1, 3, and 4 ng ml^{-1}, respectively.

5. Conclusion

A gradient, reverse-phase, HPLC method was developed to analyze indinavir, its lactone degradate, potassium sorbate, methylparaben, and propylparaben in aqueous, pediatric Xanthan Gum and NanoSystems suspensions containing Magnasweet®, povidone, hydroxypropyl cellulose, and a proprietary orange flavoring. The method has been validated and is precise and accurate. The assay has been successfully applied to Xanthan Gum suspensions and NanoSystems suspensions for human clinical and stability studies.

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