pharmaceutics

MDPI

Supplementary Materials: Optimization and evaluation of the *in vitro* permeation parameters of topical products with non-steroidal anti-inflammatory drugs through Strat-M® membrane

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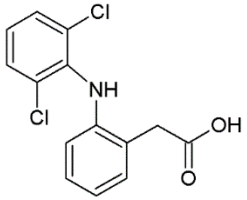
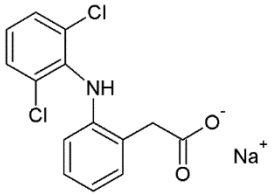
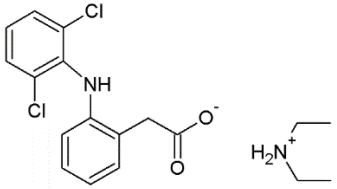
Table S1. The selected physicochemical and biological properties of the studied NSAIDs.

Active pharmaceutical ingredient (API)	Structure / formula	Molar mass [g·mol^{-1}]	Log P (experimental; octanol / water)	pK$_a$ at 25°C	Solubility in water at 25°C [mg L^{-1}]	IC 50 (COX-2) [μmol L^{-1}]
Diclofenac	 $C_{14}H_{11}Cl_2NO_2$	296.1	4.0 [1] 4.4 [2-5] 4.2 [6] 4.5 [7-9]	3.80 [3,4,10] 4.18 [11] 4.0 [12] 4.50 [2]	17.8 [1,10] 3.5 [12,13]	COX-1: 0.5, 0.3; COX-2: 0.038, 0.002 [14]; COX-2: 0.003 ± 0.001 [15]
Diclofenac sodium	 $C_{14}H_{10}Cl_2NO_2Na$	318.1	0.7 [7,16]		19 100 [17] 32 400 (37°C) [18]	
Diclofenac diethylamine	 $C_{18}H_{22}Cl_2N_2O_2$	369.3	0.853 (octanol/buffer pH=7.4) [19]		4 062.3 [10] 12 925.5 (20 °C) [10,20]	

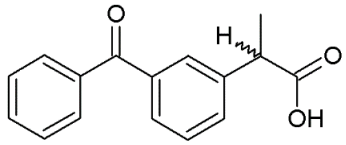
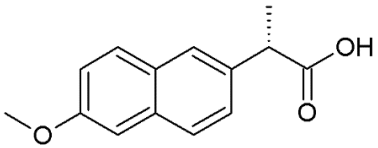
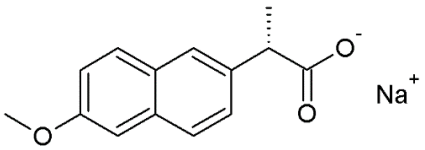
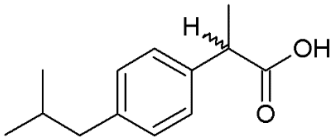
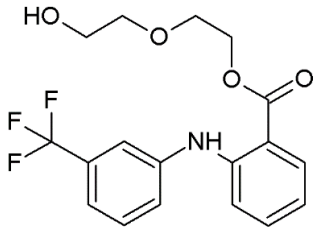
Ketoprofen	 $C_{16}H_{14}O_3$	254.3	3.12 [2,4] 1.93 [21] 3.2 [22]	4.5 [23] 4.6 [2,4] 4.39, 4.45, 4.76 [22]	210 [24] 880 (37ºC) [23] 10 (22-24 ºC) [22,25] 253 (37ºC) [22]	COX-1: 61, 5.; COX-2: 2.9, 0.24 [14]; COX-2: 0.74 ± 0.37 [15]
Naproxen	 $C_{14}H_{14}O_3$	230.3	3.18 [4,25] 2.16 [21] 3.34 [2]	4.2 [23,26] 4.6 [4] 4.15 [2]	20 [24] 65.4 [27] 17 (ambient temperature) [28] 25 [29]	COX-1: 3.0, 3.8; COX-2: 28, 35 [14]
Naproxen sodium	 $C_{14}H_{13}O_3Na$	252.1			181 200 (22.9 ºC) [30] 200 000 [31]	
Ibuprofen	 $C_{13}H_{18}O_2$	206.3	3.50 [2,4] 2.41 [21]	5.3 [23] 4.55 [4] 5.20 [2]	100 [23]	COX-1: 0.9, 2.6; COX-2: 7.2, 20 [14]
Etofenamate	 $C_{18}H_{18}F_3NO_4$	369.3	about 5 [32]	pKa1=6; pKa2=7 [33] Flufenamic acid: about 4 [32]	Etofenamate is sparingly soluble in aqueous buffers (water solubility = 9.36 mg/L based on information at https://www.drugbank.ca/drugs/DB08984). For maximum solubility in aqueous buffers, etofenamate should be dissolved in alcohol and then diluted with the aqueous buffer of choice. Etofenamate has a solubility of approximately 100 mg/L in a 1:5 solution of ethanol : PBS (pH=7.2) using this method (https://www.caymanchem.com/pdfs/23674.pdf).	Flufenamic acid: COX-1: 3.1; COX-2: 9.3 [14]

Table S2. UHPLC method parameters: sample injection volumes, retention times, and NSAIDs analytical wavelengths.

API	**Injection volume [μL]**	**Retention time [min]**	**Analytical wavelength [nm]**
Diclofenac sodium	1.0	1.9	276
Etofenamate	0.5 (gel and spray)	2.8	286
Ibuprofen	2.0	2.0	229
Ketoprofen	1.0 (gel) and 0.5 (spray)	1.0	227
Naproxen	2.0	1.5	330

Table S3. The permeation test results of topical diclofenac medications and HPLC methods for its quantification in receptor fluids.

API	Formulation	Penetration studies	HPLC assay	References
Diclofenac sodium	Carbopol® 940 gel, o/w microemulsion, Voltaren Emulgel	Franz diffusion cells PS: 1.76 cm^2; MT: Swiss albino rats skin; RF: 20 mL of phosphate buffer (pH 7.4) ; TEMP: 37°C SW: 1 g; RFA: 0.7 mL; TT: 8h; 600 rpm	---	[17]
Diclofenac epolamine	microemulsion, Poloxamer Microemulsion based gel, Flector® gel	Franz diffusion cells PS: 4.9 cm^2; MT: rat skin; RF: 300 mL phosphate buffer (pH 7.4) ; TEMP: 37°C; SW: 1.0 g (1.3%); RFA: 4.0 mL; TT: 8h; 600 rpm	--- AW: UV 276 nm	[34]
Diclofenac sodium	laminated adhesive diclofenac containing patches (including capsaicin and hyoscyamine) or only with capsaicin and hyoscyamine	Franz diffusion cells PS: 10.0 cm^2; MT: rat skin; RF: PEG 300 solution in water (1:4); TEMP: 37°C; SW: ---; RFA: 0.6 mL; TT: 24 or 48h 0.1 ml of IS solution was added to the samples before injection onto the column	Glass column Separon SGX C-18 (150_3.3 mm I.D., 7 mm) (Tessek, Prague). MP: methanol:phosphate buffer pH 6.5 (110:100) with an addition of tetrabutylammonium iodide (5 mM:l), FR: 0.5 ml/min, AW: 284 nm RT: ~3.5 min	[35]
Ionized and nonionized diclofenac	suspension or solutions in McIlvaine buffer/ethanol (0-40%)	Franz diffusion cells PS: 0.785 cm^2; MT: rat skin; RF: 3 mL phosphate buffer (pH 7.2) (10-fold of solubility); TEMP: 37°C; SV: 3 mL; RFA: 0.02 mL; TT: 8h	HPLC apparatus (Model 655, Hitachi Ltd) equipped with a variable wavelength UV monitor. Column: YMC Packed A-302 S-5 120A ODS 4.6 X 1.50 mm (Yamamura Chemical Laboratories Co., Ltd). MP: 0.1% aqueous phosphoric acid-methanol (1: 4 v/v), FR: 1.0 ml/min, AW: 283 nm	[36]
Diclofenac potassium	tablets	---	Phenomenex LUNA C18 (25 cm x 4.6 mm i.d., 5 µ) column MP: acetonitrile: sodium dihydrogen orthophosphate (adjusted to pH 3.5 using orthophosphoric acid) in the ratio of 70:30 v/v, FR: 1.0 mL/min, AW: 278 nm, RT: 9.4 min	[37]
Diclofenac sodium	propylene glycol, water, ethanol (saturated or unsaturated solution)	PAMPA plate PS: 280µl cm^2; MT: Strat-M™; RF: 280µl Krebs–Ringer bicarbonate buffer spiked with dextrose (Sigma–Aldrich, St. Louis, MO) and bovine serum albumin (4.5%, w/v; Fisher Scientific, Fair Lawn, NJ) ; TEMP: 37°C; SV: --- ; RFA: 0.02 mL; TT: 5h	---	[38]
Diclofenac sodium	suppositories	---	Lichrospher 100 RP 18 (5 µm, 25 × 4 cm; Merck). MP: methanol and phosphate buffer pH 2.5 (76:24, v/v), FR: 1.0 mL/min, AW: 254 nm, RT: ~7 min	[39]
Diclofenac sodium	tablets	---	Zorbax SB n C 18 column 150 ◊ 4.6 mm MP: methanol and water (60:40, v/v), FR: 1 mL/min, AW: 248 nm, RT: 2.73 min	[40]
Diclofenac	---	---	C-18 analytical reversed phase column (250mm×4.6mm i.d., 5µM particle size, Beckman, Palo Alto, CA, USA) MP: acetonitrile: sodium acetate buffer (75 mM, pH adjusted to 5.0 with acetic acid) in a ratio of 2:1.5 (v/v), FR: 0.5 ml/min, AW: 280 nm, RT: 16.2 min	[41]

Diclofenac diethylamine	nanoemulsion gel	---	Hypersil BDS-5 mm, RP-C8 column (250mm×4.6 mm, Thermo Scientific), MP: methanol and water (70:30 v/v) pH = 3.0±0.5 FR: 1 mL/min, AW: 280 nm, RT: 9.5 min	[42]

PS – Penetration Surface; MT – Membrane Type; RF – receptor Fluid; SW – Sample Weight; SV – Sample Volume; RFA – receptor Fluid Aliquot; TT – Total Time; MP – Mobile Phase; FR - Flow Rate; AW – Analytical Wavelength; RT – Retention Time

Table S4. The permeation test results of topical ketoprofen medications and HPLC methods for its quantification in receptor fluids.

API	Formulation	Penetration studies	HPLC assay	References
Ketoprofen	adhesive TDDs (transdermal drug delivery system)	Modified Franz diffusion cells PS: 0.951 cm^2; MT: cellophane membrane; RF: phosphate buffer (pH 7.4); TEMP: 32°C; SV: ---; RFA: 1.0 mL; TT: 7h;	---	[43]
Ketoprofen	isopropyl myristate solution	---	Phenomenex Luna C18 5µm column (250x4.6 mm) with a Luna 5-pm guard Column (30x4.6 mm) MP: acetonitrile-methanol-water (36:54:10, v/v/v), FR: 1.2 mL/min, RT: 2.3 min, AW: 265 nm	[44]
Ketoprofen	sodium alginate gel (2.5%)	Franz diffusion cells PS: 2.5 cm^2; MT: regenerated cellulose, Strat-M™; RF: phosphate buffer (pH 5.6 and 7.4) 125.0 mL; TEMP: 37°C; SW: 5.0 g; RFA: ---; TT: 24h;	---	[45]
Ketoprofen	KP-TDDS	Franz diffusion cells PS: 1.7679 cm^2; MT: full thickness rat skin; RF: phosphate buffer (pH 7.4) 12.0 mL; TEMP: 32°C; SW: ---; RFA: 500 µl; TT: 30h;	Nucleosil 100–5 C18 column (5 µm, 250x4.6mm) temp. = 40°C, MP: acetonitrile/pH 3.5 phosphate buffer mixture (55:45 v/v), FR: 1.0 ml/min, RT: 2.73 min, AW: 254 nm	[46]
Ketoprofen	benzyl alcohol / ethanol / Solutol®HS 15 / water nanoemulsion	Franz diffusion cells PS: 1.77 cm^2; MT: mouse skin; RF: phosphate buffer (pH 7.4) 11.5 mL; TEMP: 37°C; SV: 2 mL; RFA: 0.2 mL; TT: 24h;	ODS column (Tosoh, Tskgel ODS-80TS, 4.6 mm i.d × 150 mm) MP: acetonitrile and pH 7.4 phosphate buffer (78:22 v/v), FR: 1 mL/min, RT: ---, AW: 258 nm	[47]
Ketoprofen	Cremophor RH40-PEG 400 microemulsions	Franz diffusion cells PS: 2.31 cm^2; MT: shed snake skin; RF: phosphate buffer (pH 7.4) PBS 6.0 mL; TEMP: 32°C; SV: ---; RFA: 1.0 mL; TT: 12h	C18 column (Waters, USA, particle size = 5µm; column dimension = 4.6 × 150 mm) FR: 1 ml/min, MP: 0.1% (v/v) phosphoric acid: methanol (25:75, v/v), AW: 254 nm	[48]

PS – Penetration Surface; MT – Membrane Type; RF – Receptor Fluid; SW – Sample Weight; SV – sample volume; RFA – Receptor Fluid Aliquot; TT – Total Time; MP – Mobile Phase; FR - Flow Rate; AW – Analytical Wavelength; RT – Retention Time

Table S5. The permeation test results of topical naproxen medications and HPLC methods for its quantification in receptor fluids.

API	Formulation	Penetration studies	HPLC assay	References
Naproxen	solution and commercial gels: Reuxen, Tecnifar, Naprosyn	Franz diffusion cells PS: 1.0 cm^2; MT: cellulose acetate and polyethersulphon; RF: phosphate buffer (pH 7.4) PBS 4.0 mL; TEMP: 32°C; SW: 1 g; RFA: 0.1-1.0 mL; TT: 24h	---	[49]
Naproxen sodium	tablets	---	RP -UFLC C18G column (250 × 4.6mm i.d., 5μm) MP: methanol:10mM tetra butyl ammonium hydrogen sulfate (80:20, v/v), FR: 1.2mL/min, RT: 4.5 min, AW: 231 nm	[50]
Naproxen	microemulsions	Franz diffusion cells PS: 3.4618 cm^2; MT: cellulose membrane; RF: phosphate buffer (pH 7.4) 25.0 mL; TEMP: 32°C; SW: 5 g; RFA: 2.0 mL; TT: 24h	--- AW: 271 nm	[51]
Naproxen	solid lipid nanoparticles	Franz diffusion cells PS: 3.4618 cm^2; MT: rat skin; RF: 50:50 ethanol/water mixture (5.5 mL); TEMP: 37°C; SV: 10 mL; RFA: ---; TT: 24h	Agilent Eclipse XDB-C18 column (5μm, 4.6 × 250 mm) MP: 40:20:40 acetonitrile, methanol and acetic acid (1% v/v), FR: 0.7 ml/min, RT: 11 min, AW: 230 nm	[52]
Naproxen	o/w microemulsions	Franz diffusion cells PS: 0.75 cm^2; MT: cellulose membrane; RF: 50:50 ethanol/water mixture (4.5 mL); TEMP: 35°C; SV: 500 μl; RFA: 200 μl; TT: 22h	Waters Symmetry, 4.6 × 15 cm reverse phase column (C18). MP: methanol/phosphate buffer pH 2.5 (65:35 v/v), FR: 1 ml/min, AW: 274 nm, RT: ---	[53]

PS – Penetration Surface; MT – Membrane Type; RF – Receptor Fluid; SW – Sample Weight; SV – sample volume; RFA – Receptor Fluid Aliquot; TT – Total Time; MP – Mobile Phase; FR - Flow Rate; AW – Analytical Wavelength; RT – Retention Time

Table S6. The permeation test results of topical ibuprofen medications and HPLC methods for its quantification in receptor fluids.

API	Formulation	Penetration studies	HPLC assay	References
Ibuprofen	methanol solution	---	Rainin Microsorb 3μm; 10 × 4.6 mm reverse phase column (C18) MP: acetonitrile, 0.1 M sodium acetate (35:65 v/v; glacial acetic acid added to obtain pH=6.4), FR: 1.5 ml/min AW: 220 nm, RT: 4.0 min, Temp.: 50°C	[54]
Ibuprofen	ointment	---	Precolumn: Pye Unicam RP-18, 10μm; 4.6 × 30 mm Analytical column: Pye Unicam RP-18, 10μm; 4.6 × 100 mm MP: THF, 0.2M NaH_2PO_4 (45:55, v/v; pH=4), FR: 1.0 ml/min, AW: 219 nm, RT: 6.6 min, Temp.: -	[55]
Ibuprofen	gel	Franz diffusion cells PS: 1.131 cm^2; MT: synthetic hydrophilic GH Polypro filter, pore size: 0.45nm; RF: phosphate buffer, pH=7.4 (6.5 mL); TEMP: 32°C; S: 1g; RFA: 500 μl; TT: 8h	C18 column, 5μm; 3.9 × 150 mm MP: acetonitrile, 0.01M H_3PO_4 (45:55, v/v), FR: 1.0 ml/min, AW: 264 nm, RT: 7.129 min, Temp.: -	[56]
Ibuprofen	gel	Franz diffusion cells PS: 1.00 cm^2; MT: excised porcine abdominal skin; RF: phosphate buffer, pH=7.4 (8.0 mL); TEMP: 32.0±0.5°C; S: -;RFA: 200 μl; TT: 8h	HyperClone reverse phase C18 column, 5μm; 4.6 × 250 mm MP: acetonitrile, phosphate buffer pH=7.4 (gradient; 30:70, 10 min), FR: 1.0 ml/min, AW: 233 nm, RT: -, Temp.: -	[57]
Ibuprofen	gel	Vertical diffusion cells PS: about 2 cm^2; MT: silicone membrane (Silatos 120 μm thickness), pig ear skin; RF: phosphate-buffered saline pH=7.4 67mM (6.5 mL); TEMP: 37°C; S: -; RFA: 200 μl; TT: 8h	Merck Lichrospher100 RP18, 5μm MP: acetonitrile, citrate buffer pH=2.4 (55:45 v/v), FR: 1.2 ml/min, AW: 227 nm RT: about 5 min, Temp.: -	[58]
Ibuprofen	microemulsion	Franz diffusion cells PS: 2.8 cm^2; MT: porcine ear skin; RF: phosphate-buffered saline pH=7.4 (7.0 mL); TEMP: 37°C; S: 1.0 g; RFA: 500 μl; TT: 8h	Merck Lichrospher C18, 5μm; 4. 6 × 250 mm MP: acetonitrile, sodium acetate buffer pH=2.5 (70:30 v/v), FR: 0.8 ml/min, AW: 264 nm, RT: about 7.5 min, Temp.: -	[59]
Ibuprofen, acetaminophen, chlorzoxazone	tablets	---	Kromasil C8, 5μm; 4. 6 × 250 mm MP: acetonitrile, 0.2% trimethylamine solution (pH=3.2 obtained with H_3PO_4) (50:50 v/v), FR: 1.5 ml/min, AW: 215 nm, RT: about 12 min, Temp.: 20±1°C	[60]
Ibuprofen	spray, gel, cream, foam	Franz diffusion cells PS: 1.15 cm^2; MT: human skin; RF: phosphate-buffered saline pH=7.4, ethanol (75:25, v/v) (2.5 mL); TEMP: 37.0±0.5°C; S: 5mg/cm^2; RFA: 200 μl; TT: 48h	RP Apex ODS column; 4. 6 × 250 mm MP: acetonitrile, buffer (60:40, pH=2.5), FR: 1.5 ml/min, AW: 225 nm, RT: -, Temp.: -	[61]
Ibuprofen	gel	Franz diffusion cells PS: 0.64 cm^2; MT: hairless mouse skin; RF: isotonic phosphate buffer pH=7.2 with 0.1% (v/v) formaldehyde and 0.5% (w/v) Brij 58 (5.1 mL); TEMP: 37.0±0.5°C; S: 200 μl; RFA: 300 μl; TT: 24h	Reverse phase C18 column, 5μm; 4. 6 × 150 mm MP: acetonitrile, monobasic sodium phosphate buffer pH=3 (60:40), FR: 1.0 ml/min, AW: 254 nm, RT: 3.8 min, Temp.: -	[62]

PS – Penetration Surface; MT – Membrane Type; RF – Receptor Fluid; S – Sample Amount; RFA – Receptor Fluid Aliquot; TT – Total Time; MP – Mobile Phase; FR - Flow Rate; AW – Analytical Wavelength; RT – Retention Time

Table S7. The permeation test results of topical etofenamate medications and HPLC methods for its quantification in receptor fluids.

API	Formulation	Penetration studies	HPLC assay	References
Etofenamate	solution in mobile phase	---	Qualisil Gold C18 column, 5μm; 4.6 × 250 mm MP: methanol, 0.2% triethylamine in water (v/v) at 85:15 ratio (v/v) (pH adjusted to 6.5 with 10% v/v H_3PO_4), FR: 1.2 ml/min, AW: 286 nm, RT: 5.3 min, Temp.: -	[33]
Etofenamate	drug solutions	---	Qualisil BDS C18 column, 5μm; 4.6 × 250 mm MP: methanol, phosphate buffer (80:20, v/v) (pH adjusted to 6.0 with H_3PO_4), FR: 1.0 ml/min, AW: 286 nm, RT: 7.5 min, Temp.: -	[63]
Etofenamate	plasma, synovial fluid and tissue samples	---	Nucleosil 7 C18 column, 5μm; 4.0 × 250 mm MP: -, FR: 1.0 ml/min, AW: 286 nm, RT: about 15 min, Temp.: -	[64]
Etofenamate	gel	Franz diffusion cells PS: 1 cm^2; MT: hydrophilic polysulfone filters, human abdominal skin; RF: ethanol, phosphate buffer pH=7.4 (2:3, v/v); TEMP: 32±2°C; SW: 0.2±0.1g; RFA: 200 μl; TT: 12h	Reversed phase Lichrospher 100 RP18 column, 5μm; 4.0 × 125 mm MP: methanol, acetonitrile, water (45:35:20, v/v/v), FR: 1.0 ml/min, AW: 286 nm, RT: -, Temp.: -	[65]

PS – Penetration Surface; MT – Membrane Type; RF – Receptor Fluid; SW – Sample Weight; RFA – Receptor Fluid Aliquot; TT – Total Time; MP – Mobile Phase;
FR - Flow Rate; AW – Analytical Wavelength; RT – Retention Time

Table S8. The validation parameters for the assays of NSAIDs by the UHPLC-UV method.

Parameter	Diclofenac sodium	Etofenamate	Ibuprofen	Ketoprofen	Naproxen
Specificity[a]	✓	✓	✓	✓	✓
Linearity range[b] [mg/mL]	0.22-1.00	0.83-4.11	0.20-0.65	0.12-0.43	0.35-1.78
Slope	5253215	2960365	1803474	9451898	1753264
Intercept	27620	-207535	96769	21058	17550
Correlation coefficient (r)	0.997	0.998	0.995	0.995	0.999
SE of slope	106529	55011	49399	249923	11159
SE of intercept	70805	149930	21420	70864	13151
LOD [mg/mL]	0.07	0.28	0.06	0.04	0.04
LOQ [mg/mL]	0.22	0.83	0.20	0.12	0.12
Stability of API in standard solution[c]	>12h	>12h	>12h	>12h	>12h
System suitability[d] [%]	0.28	0.30	0.21	0.21	0.21
Interday precision[e] [%]	0.39	1.75	1.10	1.74	0.50
Intraday precision[f] [%]	0.90	2.44	1.27	2.28	1.24
Accuracy (recovery)[g] [%]	100.4±3.8	100.8±3.9	98.7±7.0	99.7±5.2	99.86±2.01

[a] the medium and placebo must not interfere with the determination of active substances,

[b] 5 relative concentrations of the solution (25 – 125% for each API, y=ax+b, r≥0,995, slope (a) – significant, intercept (b) – irrelevant),

[c] the relative difference in peak surface area values for the standard and test solution immediately after preparation and after "t" from preparation should not be greater than 2% (RSD ≤ 2%),

[d] based on 6 injections of standard solution with concentration corresponding to 100% of API (RSD standard for n=6 ≤ 2%),

[e] based on analysis of 6 samples of standard solution with concentration corresponding to 100% of API from 1 Vertical Diffusion Cell (RSD for one group of results ≤ 5%),

[f] based on 2 groups of results done by 2 analysts, where one group of results is based on analysis of 6 samples of standard solution with concentration corresponding to 100% of API from 1 Vertical Diffusion Cell by 1 analyst (RSD for one group of results ≤ 2%, the relative difference between means ≤ 5%, coefficient F calc.< F crit. (F crit.=5.05)),

[g] 5 relative concentrations of the solution (25 – 125% for each API, the average recovery value must be in the range of 95 - 105%, each result must fall within the specified confidence interval (average recovery value ± 2SD)).

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