QUANTITATIVE DETERMINATION OF AMIKACIN IN THE SKIN: EXTRACTION, DERIVATIZATION AND HPLC ANALYSIS

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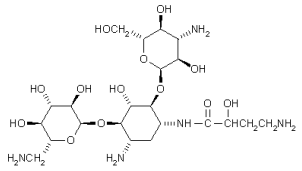
OBJECTIVE

To develop and validate an assay procedure for amikacin (AK) extraction and quantification in rabbit epidermis and dermis after in vitro transdermal transport experiments.



METHODS

HPLC Analysis

- ✓ Isocratic HPLC pump
- ✓ 10 µm µBondapack 300 X 4.6 mm (Waters)
- ✓ T = 45°C
- ✓ Eluent: $CH_3CN:H_2O$ (47:53 v:v) + CH_3COOH 1ml/L
- ✓ Flow: 1.5 ml/min
- ✓ Injected volume: 100 µl
- ✓ λ = 365 nm

Each solution was derivatized separately

Derivatization

Derivatisation	µl
AK Acqueous solution	100
MeOH	200
NaOH 0.05 N	40
1-fluoro-2,4-dinitrobenzene 180 mg/ml in MeOH	50

Conditions: 90°C; 10 minutes

Permeation/Accumulation Experiments

- oFranz-type diffusion cell
- o Rabbit ear skin
- o Receptor solution: 0.9% NaCl
- o Donor:
- • Commercial Gel (Likacin) 5% AK
- • AK Solution 50 mg/ml pH 7.4
- • AK Solution 50 mg/ml pH 4

Epidermis-Dermis separation

- •Heat (hair-dryer; 20 s)
- •Separation
- •Weighting of epidermis and dermis

AK extraction from epidermis and dermis

RESULTS

Derivatization and HPLC validation

Nominal Value (µg/ml)	Fitted Value	SD	n	RSD%	ER %
1.64	1.60	0.02	6	2.2	2.70
4.10	3.99	0.07	3	2.15	2.68
8.20	7.76	0.28	6	3.89	5.41
16.40	16.23	0.06	8	0.4	1.04
32.81	32.74	0.42	9	1.31	1.11
49.21	49.65	0.10	3	0.2	0.90

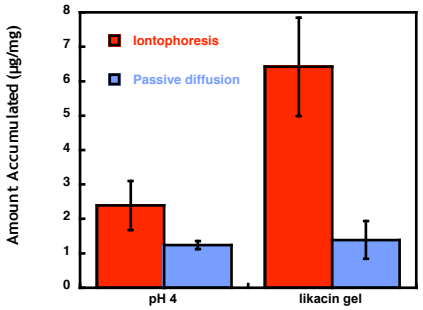
Nominal value, fitted value (y=63737x-35784), RSD% and ER% calculated for each concentration tested

AK extraction recovery from Epidermis and Dermis

Extraction Solvent	Volume	T	% Recovery EPIDERMIS	% Recovery DERMIS
MeOH: H_2O (50:50)	250 µl	45°C	77.6 ±28.3	15.5 ± 9.6
MeOH: H_2O (50:50)	500 µl	45°C	66.4 ± 14.6	26.6 2 ± 24.9
MeOH: H_2O: NaOH 0.05M (5:5:2)	500 µl	45°C	67.3 ± 7.2	53.9 ± 16.3
MeOH: H_2O: NaOH 0.05M (5:5:2)	500 µl	60°C	92.9 ± 1.1	n.d.?

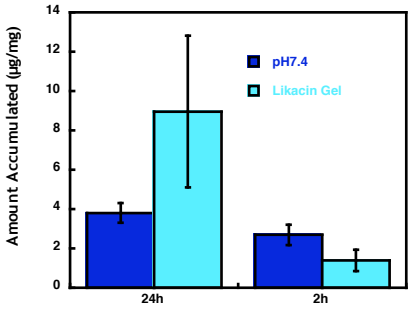
Accumulation of AK in epidermis

(2 hours of passive diffusion or anodal iontophoresis 0.5 mA/cm²)



Accumulation of AK in epidermis after

(passive, 2 and 24 hours of application)



Amount of AK permeated

	Application Time (h)	Ionto	Amikacin permeated (µg/cm²)	s.e.m.
Commercial gel[a]	24	N	463.56	289.97
Solution pH 7.4[b]	24	N	201.6	152.26
Solution pH 7.4[b]	2	N	12.09	4.24
Commercial gel[a]	2	N	2.45	3.22
Commercial gel[a]	2	Y	781.16	98.14
Solution pH 4[b]	2	Y	354.14	148.32
Solution pH 4[b]	2	N	1.03	0.04

CONCLUSION

- ✓The extraction, derivatization and HPLC assay has good reproducibility, sensitivity and specificity resulting in a reliable method for biopharmaceutical studies of AK distribution in epidermis.
- ✓For the dermis, the recovery efficiency needs to be improved
- ✓Despite its high molecular weigh, amikacin is able to cross the skin and to accumulate in the epidermis and dermis
- ✓The application of iontophoresis (2 h) is able to greatly enhance the amount permeated (EF>300), envisaging the possibility of an iontophoretic transdermal administration of this drug.