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Comment on activity of commercial streptokinase preparations: issue of sub-standard life-saving drugs

Prasanta K. Ghosh and Bakulesh M. Khamar

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Letters to the Editor

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Comment on activity of commercial streptokinase preparations: issue of sub-standard life-saving drugs

We read the pre-clinical research paper of Hermentin *et al.*[1] with interest. The paper compares the potency and purity of different commercial streptokinase (SK) preparations.

The paper, inter alia, analyses STPase Batches 2008 and 2009 manufactured by Cadila Pharmaceuticals Ltd (CPL), India.

STPase is a natural SK produced by *Streptococcus* ATCC 12449 and is not a recombinant product as has been mentioned in the paper. Amino acid sequence of STPase is well characterized and matches with authentic SK. Results of N-terminal sequence analysis of the STPase carried out in four different experiments are shown in *Table 1*.

The STPase was also analysed by trypsin digestion followed by RP-HPLC for mapping. N-terminal sequences of six tryptic-digests peptides match completely with the natural SK protein sequence.[2]

The euglobulin assay carried out in February 2004 with the earlier mentioned batches gave the potency of 14.75×10^5 and 14.32×10^5 IU per vial, which was 98.3 and 95.5% of the declared potency, respectively. In India, Streptase and STPase are sold as per Indian Pharmacopeia (IP). Estimation of SK potency is carried out by euglobulin clot lysis assay instead of chromogenic method. By chromogenic assay, the potency was >100%. The latter method gives higher potency than that obtained from euglobulin assay.

The details of the procedures adopted for preparing STPase samples for activity determination have not been elaborated by Hermentin *et al.*[1] On reconstitution, all SK samples lose activity with time. Samples prepared and left for long time even at −15 to −25°C preservation on thawing have shown large difference in the activity.[3] Reconstituted Streptase lost substantial activity (>60% after 24 h) when preserved[3] at 4°C.

Lyophilized SK requires storage at 2–8°C and cold chain integrity maintenance is essential to preserve the quality of the product. The history of transport and handling the STPase batches is not mentioned. It is not unlikely that improper conditions of exposure of STPase batches has led to the observed results and this has also been recognized by the authors in the paper.

STPase is manufactured by CPL based on the technology developed by the Institute of Microbial Technology, Chandigarh, a national institute under the Indian Council for Scientific and Industrial Research, New Delhi, a research organization of the government of India.

Table 1 N-terminal sequencing analysis performed on STPase

Experiment	Sequence	Residue number in SK
I	IAGPEWLL	1–8
II	IAGPE	1–5
III	IAGPE	1–5
IV	IAGPEWLLDRP-SVNNSQLVVSVA-GTVEGTN	1–30

References

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Prasanta K. Ghosh
Cadila Pharmaceuticals Ltd
Sarkhej-Dholka Highway
Bhat
Ahmedabad 382210
India
E-mail address: pghosh@cadilapharma.co.in

Bakulesh M. Khamar
Cadila Pharmaceuticals Ltd
Sarkhej-Dholka Highway
Bhat
Ahmedabad 382210
India

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Comment on activity of commercial streptokinase preparations: issue of substandard life-saving drugs: reply

In our paper, 'Comparative analysis of the activity and content of different streptokinase preparations,'[1] we only mentioned the results of the chromogenic assay for the activity measurement of STPase. To confirm, we also tested the samples with a clot-lysis assay as second assay. We have not shown the data of this confirmational tests as they did not significantly differ from the results of the chromogenic assay. The results of both assays are listed in *Table 1*. As the fibrinolytic activity detected in these two samples was that low, we tested a third batch in a second run, but found no significant difference to the previously tested samples as also shown in *Table 1*.

We performed the tests with material directly drawn from the original containers and performed measurements immediately after appropriate dilution. Moreover, the results of our activity tests have also been confirmed by independent investigators.[2] Ghosh and Khamar stated in their letter that the detected low activity may be a result of advanced degradation due to inadequate storage or transportation. As previously mentioned, we kept the products, bought for this survey, under the conditions required by the respective manufacturers. This does not exclude a degradation of this product prior to our sample collection. Ghosh and Khamar stated in their letter that the respective streptokinase is remarkably sensitive vs. environmental influences. This may be a cause that we were in fact not able to detect even traces of the original protein. However, in our experience, it is possible to stabilize streptokinase in a way that transportation and storage are not an issue as addressed in the letter, but can be done at elevated temperatures, e.g. at room temperature without significant loss of fibrinolytic activity over time.

In conclusion, we are confident with the results measured for the respective samples in our laboratories, although as stated in our publication as well as in the letter of Ghosh and Khamer, the origin of the detected deficiencies remains unknown.

Table 1 Activity of three batches of STPase measured with a cromogenic assay (method published in reference 1) and a clot-lysis assay as described in the European Pharmacipoeia

Batch number	Chromogenic assay (%)	Clot-lysis assay (%)
2008	312 075 IU (20.8)	297 410 IU (19.8)
2009	349 650 IU (23.3)	337 440 IU (22.5)
3028	264 375 IU (16.7)	264 375 IU (17.6)